



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION IX
75 Hawthorne Street
San Francisco, CA 94105

August 29, 1994

Steven L. Costa
Project Manager
CH2M Hill
P.O. Box 12681
Oakland, CA 94604-2681

Re: Comments on Bioassay Testing of Ocean Disposed High-Strength
Waste of StarKist Samoa, Inc. and VCS Samoa Packing Company

Dear Steve:

We have reviewed the report of June 29, 1994 for the first of three rounds of bioassays of high-strength waste, as required by the canneries' ocean disposal permits. The report is based on two sampling events: the first was collected on February 16, 1994; and, a second sample was required and tested in March 1994, due to test failure of the echinoderms in the first sample. Your proposed changes to the study methods, as outlined in your memo of July 1, 1994, are acceptable. Enclosed is a memo from Amy Wagner of EPA's Laboratory Support Section, detailing the acceptable changes. Please call Amy at (510) 412-2329 if you have any questions on her comments.

We note that the second and third rounds of testing were scheduled for May and August 1994, and we would like to know if these tests were conducted as scheduled and, if not, the rescheduled dates, and when we can anticipate the reports on these bioassays. Please relay this information to Pat Young, American Samoa Program Manager, or if you have any questions, call her at (415) 744-1594.

Sincerely,


Norman L. Lovelace, Chief
Office of Pacific Island and Native
American Programs (E-4)

Enclosure

cc: Jim Cox, Van Camp Seafood Company
Norman Wei, StarKist Seafood Company
Tony Tausaga, American Samoa EPA
Sheila Wiegman, American Samoa EPA
Allan Ota, W-3-3
Amy Wagner, P-3-1



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION IX LABORATORY
1337 S. 46TH STREET BLDG 201
RICHMOND, CA 94804-4698

AUG 22 1994

MEMORANDUM

SUBJECT: Review of Bioassay Testing of Starkist, Samoa, Inc. and VCS Samoa
Packing High Strength

FROM: *AW for ALW*
Amy Wagner
Laboratory Section (P-3-1)

THRU: *Brenda Bettencourt*
Brenda Bettencourt, Chief
Laboratory Section (P-3-1)

TO: Pat Young
OPINAP (E-4)

Allan Ota
Wetlands and Sediment Management Section (W-3-3)

At your request, I have reviewed "Results of a Bioassay Conducted on Two High Strength Waste Samples from the Van Camp and Starkist Tuna Canneries in American Samoa." The following recommendations are based on the results of the first round of testing.

1. p. 11. The salinity of the *Mysidopsis bahia* tests were 25 ppt, presumably based on the salinity of the shipping water. An effort should be made to find a supplier that raises mysids in a salinity closer to that of the discharge site, between 30-35 ppt.
2. Appendix, p. 1. It is recommended that the water quality measurements pH, dissolved oxygen, and initial salinity be measured for all samples upon receipt.

3. Appendix, Table 10. The salinities of 26-28 ppt most likely caused the high mortality in controls with the sea urchin toxicity test. If necessary, brine adjustments should be used to increase the salinity of test samples to the test method requirements of 30 ± 2 ppt.

4. To reduce salinity elevation throughout the tests, an attempt should be made to cover test containers to reduce evaporation.

Based on the results of these tests, the following changes in the bioassay methods recommended by CH2M Hill in the cover memo are acceptable.

1. The series of the concentrations for toxicity tests can be reduced to 2.0%, 1.0%, 0.5%, 0.25%, 0.125%, and 0.0625% instead of the suggested series.

2. *Mytilus edulis* can be used instead of *Strongylocentrotus purpuratus* as the third test organism. The oyster *Crassostrea virginica* may be substituted for the mussel test during the months when mussels cannot be spawned.

3. Aeration should be provided in the mussel test containers due to high biological oxygen demand of the effluent. In addition to a control with aeration, a control without aeration should be run. A t-test should be used to determine if there is any significant effect of aeration.

Any questions on the comments can be addressed to me at (510) 412-2329.

cc: Jeff Rosenbloom, Chief
Wetlands and Sediment Management Section (W-3-3)

Copy to Alan
Ota, ASEP A

MEMORANDUM

CH2M HILL

TO: Pat Young/USEPA

COPIES: Amy Wagner/USEPA (w/ attachments)
Kurt Kline/ABT (w/o attachments)

FROM: Steve Costa/CH2M HILL/SFO
Don Kingery/CH2M HILL/SFO

DATE: July 1, 1994

SUBJECT: Bioassay Testing of Starkist Samoa, Inc. and VCS Samoa Packing High Strength Waste

PROJECT: OPE030702.EL.R2



High strength waste (HSW) bioassays are required by Special Condition 3.3.5 of Starkist Samoa's and VCS Samoa Packing's ocean dumping permits. The results of the tests are presented in the attached: "*Results of a Bioassay Conducted on Two High Strength Waste Samples from the Van Camp and Starkist Tuna Canneries in American Samoa*" prepared by Advanced Biological Testing Inc., Tiburon, California.

Acute effluent bioassays were conducted on *Mysidopsis bahia* (mysid shrimp) juveniles, *Mytilus edulis* (blue mussel) larvae, *Strongylocentrotus purpuratus* (purple sea urchin) larvae, and *Citharichthys stigmaeus* (speckled sanddab) juveniles using HSW collected separately from the Starkist Samoa and VCS Samoa Packing canneries in Pago Pago Harbor, American Samoa. The results of these bioassays are summarized in the table below.

Based on the results of the bioassays, we recommend the following changes to the HSW bioassay protocol:

Reduce the upper end of the HSW concentration series for all bioassays to a maximum of 3.0%. The results of the bioassay tests give a better understanding of the test concentrations needed. No additional information is required at concentrations greater than 3.0%. Reducing the maximum concentrations will reduce the amount of HSW that needs to be sampled and shipped. We recommend a series of concentrations for the bioassays of 3.0%, 1.5%, 0.8%, 0.2%, 0.1%, and 0.05%.

Continue running bioassays with *Mytilus edulis* while monitoring the effects of aeration on organism mortality but drop the use of *Strongylocentrotus purpuratus* larvae as test organisms for the HSW. This recommendation is made for the following reasons:

- Special Condition 3.3.5 of the permits requires only three organisms be tested; one organism each out of three specified groups. *Mysidopsis bahia*

MEMORANDUM

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July 1, 1994

OPE030702.EL.R2

and *Citharichthys stigmaeus* satisfy the requirements for Groups 2 and 3. Group 1 contains larval stages of both bivalves and echinoderms and running just *Mytilus edulis* should satisfy this requirement.

- Because of the high oxygen demand of the effluent, all test containers required aeration throughout the tests to maintain adequate oxygen concentrations for the test organisms. Aerating the chambers using *Mytilus edulis* and *Strongylocentrotus purpuratus* larvae as bioassay test organisms gives problematic results. Aeration is standard protocol for bioassays on fish and invertebrates when oxygen levels fall below 40% of saturation, but is not standard protocol for bioassays on larval bivalves and echinoderms. The effects of aerating the water on the survival of these organisms is not known. Because the *Mytilus edulis* bioassays are only run for two days (vs. four for the *Strongylocentrotus purpuratus*) the organisms are exposed for half the time and the effects of aeration may be reduced.
- The mortality of the control group was substantial for the echinoderms and is unacceptable according to protocol. The cause of the high mortality in the control is not known at this time.

Please review the above recommendations. We suggest Amy Wagner contact Kurt Kline, Advanced Biological Testing Inc., directly at (415)435-7878 to discuss any comments you have on the bioassay protocols.

Summary of High Strength Waste Bioassay Results.				
Test Organism	Starkist Samoa		VCS Samoa Packing	
	LC ₅₀	NOEC/IC ₅₀ ¹	LC ₅₀	NOEC/IC ₅₀ ¹
<i>Citharichthys stigmaeus</i> (sanddab)	0.27%	0.2%	0.59%	0.4%
<i>Mysidopsis bahia</i> (mysid shrimp)	0.12%	0.05%	0.59%	0.05%
<i>Mytilus edulis</i> (blue mussel)	> 1.2%	< 0.08%	> 1.2%	< 0.08
<i>Strongylocentrotus purpuratus</i> ² (urchin)	> 1.2%	< 0.08%	> 1.2%	0.1%
¹ NOEC reported for the juvenile sanddabs and mysid shrimp, IC ₅₀ reported for the mussel and urchin larvae.				
² Control survival of 64.4% is unacceptable according to protocol.				

**RESULTS OF A BIOASSAY CONDUCTED ON
TWO HIGH STRENGTH WASTE SAMPLES
FROM THE VAN CAMP AND STARKIST TUNA CANNERIES
IN AMERICAN SAMOA**



**Advanced
Biological
Testing Inc.**

**RESULTS OF A BIOASSAY CONDUCTED ON
TWO HIGH STRENGTH WASTE SAMPLES
FROM THE VAN CAMP AND STARKIST TUNA CANNERIES
IN AMERICAN SAMOA**

Prepared for:

CH2M Hill California, Inc.
1111 Broadway
Oakland, CA 94607
Project # PDX 30702

Prepared by:

Advanced Biological Testing Inc.
98 Main St., # 419
Tiburon, Ca. 94920

June 29, 1994

Ref: 9309-2

INTRODUCTION

At the request of CH2M Hill (Project # PDX 30702), Advanced Biological Testing conducted acute effluent bioassay testing on *Mysidopsis bahia*, *Mytilus edulis*, *Strongylocentrotus purpuratus* and *Citharichthys stigmaeus* using high strength wastes (HSW) collected separately from the Van Camp (HSW-1) and Starkist (HSW-2) tuna canneries in American Samoa. The study was run using methods generally specified in EPA 1991 and in a Sampling and Testing Plan submitted to the EPA.

The study was conducted at the Advanced Biological Testing Laboratory in Tiburon, California, and was managed by Mr. Mark Fisler.

2.1 EFFLUENT SAMPLING

The high strength wastes were sampled as composites on February 16, 1994 by personnel from CH2M Hill. Due to shipping and airline scheduling problems, frequently encountered in this region, the sample was received by the laboratory on February 19, 1994. Two five gallon carboys were provided from each cannery defined as HSW-1 (VCS) and HSW-2 (SK) and were maintained in ice-filled coolers from the date of sampling until laboratory receipt. The sample were at 2-3°C upon receipt.

Due to the test failure in the echinoderms, both of the HSW were resampled on March 30, 1994, and shipped to ABT arriving on April 4, 1994.

2.2 SAMPLE PREPARATION

2.2.1 Testing on the speckled sanddab, *Citharichthys stigmaeus*

After extensive discussions with the EPA regarding the proposed testing concentrations, the high strength wastes were tested at eight concentrations starting from 3.0% and dropping using a 50% dilution factor. The final concentrations were 3.0, 1.5, 1.25, 0.8, 0.4, 0.2, 0.1 and 0.05% as vol:vol dilutions in seawater. The diluent was filtered seawater from the Bodega Bay Marine Laboratory. The dilutions were brought up to the test temperature (14°C) and aerated continuously. Based upon data provided by CH2M Hill, and subsequently supported by information from the EPA, these effluents have an extremely high biological oxygen demand, therefore aeration was carried out from the beginning of the test.

A reference toxicant was run using concentrations of the toxicant Sodium Dodecyl Sulfonate (SDS) made up as a 2 grams per liter stock solution in distilled water. The tested concentrations were set at 25, 12.5, 6.25, 3.1, and 1.6 mg/L in 30 ppt seawater in a 24 hour test.

2.2.2 Testing on the mysid, *Mysidopsis bahia*

Both of the high strength wastes were tested twice, once in a concentration series of 25, 12.5, 6.25, 3.1, 1.6, 0.8, and 0.4% vol:vol in seawater, and after discussions with the EPA, a second

time at a lower concentration series of 1.6, 0.8, 0.4, 0.2, 0.1 and 0.05% vol:vol dilutions. The diluent was filtered seawater from the Bodega Bay Marine Laboratory. The dilutions were brought up to the test temperature (20°C) and aerated continuously.

A reference toxicant was run using concentrations of the toxicant Sodium Dodecyl Sulfonate (SDS) made up as a 2 grams per liter stock solution in distilled water. The tested concentrations were set at 20, 10, 5, 2.5 and 1.25 mg/L in 30 ppt seawater in a 96 hour test.

2.2.3 Echinoderm and Bivalve Larval Bioassay

Test solutions used in the bioassays were prepared using San Francisco Bay seawater at 28 ppt in serial dilution (0.5) to create 0.08%, 0.15%, 0.3%, 0.6% and 1.2% test concentrations for the bioassays. The echinoderm test failed control survival in two testing attempts using the initial HSW delivered on February 19, 1994. A second sample was requested from each cannery which was delivered on April 4, 1994. The echinoderm test again marginally failed the controls and the results of the study are presented for information. The bivalve study conducted concurrently with the echinoderm bioassay passed the control criteria.

The reference toxicant for the echinoderm and bivalve larval bioassays was copper at test concentrations of 0.56, 3.2, 10, 32, and 56 µg/L.

2.2.4 *Citharichthys stigmaeus*

The bioassays were carried out on juvenile *Citharichthys stigmaeus*, supplied by J. Brezina and Associates in Dillon Beach, California. The animals were received at ABT on February 19, 1994. The test conditions are summarized in Table 1. Five replicates of each concentration were tested with ten juvenile fish per replicate. Water quality was monitored daily as initial quality on Day 0 and final water quality on Days 1-4. Parameters measured included dissolved oxygen, pH, salinity, total ammonia, and temperature.

2.2.5 *Mysidopsis bahia*

The first bioassay was carried out on 7-10 day old larval *Mysidopsis bahia*, supplied by J. Brezina and Associates in Dillon Beach, California. The animals were received at ABT on February 19, 1994. The test conditions for this test are summarized in Table 2. The second test was carried out on larval mysids supplied by Aquatox from Hot Springs, Arkansas. The animals

were received at ABT on February 26, 1994. The test conditions for the second test are summarized in Table 3.

Five replicates of each concentration were tested with ten larval mysids per replicate. Water quality was monitored daily as initial quality on Day 0 and final water quality on Days 1-4. Parameters measured included dissolved oxygen, pH, salinity, total ammonia, and temperature.

2.2.6 Echinoderm Larval Development Test

The echinoderm larvae survival and development test followed draft ASTM methods (ASTM, 1994). Purple urchins, *Strongylocentrotus purpuratus*, were obtained from A. K. Siewers, Santa Cruz, California. Adults were induced to spawn by intercoelomic injection of 0.5M KCl. Released eggs were placed in individual containers of filtered seawater, and sperm was collected dry and held on ice. Gametes were mixed and allowed to fertilize for up to two hours. Fertilized eggs were then separated from sperm and debris by filtering the suspension at 20 μ m. Egg stock density was estimated by counting an aliquot of dilute stock concentrate. Equal volumes of concentrate were added to each replicate to an initial density of 15-30 embryos per mL. Initial stocking density was confirmed by counting a 5 mL aliquot from at least three control replicates.

Testing was conducted at $16 \pm 2^{\circ}\text{C}$ under a 14 hour light and 10 hour dark photoperiod. Temperature, pH, dissolved oxygen, and salinity were recorded at 0, 24, 48 and 72 hours in water quality replicates. Total ammonia was measured in the 1.2% sample at 0 and 48 hours. At the end of the exposure period, a 5 mL sub-sample was taken from each test replicate and preserved with buffered formalin. Sub-samples were counted in a Sedgwick-Rafter cell, and the total number of normal and abnormal larvae were counted.

2.2.7 *Mytilus edulis* Larval Survival and Development Test

The bivalve larvae survival and development test was run in parallel with the echinoderm using the second set of effluents. The test followed methods in ASTM (1993). Bay mussels, *Mytilus edulis*, were obtained from A. K. Siewers, Santa Cruz, California. Adults were induced to spawn by heat shocking. Released gametes were placed in individual containers of filtered seawater and examined for viability. Gametes were mixed and allowed to fertilize for up to two hours, under gentle aeration. Fertilized eggs were then separated from sperm and debris by filtering the suspension at 20 μ m. Egg stock density was estimated by counting an aliquot of dilute stock concentrate. Equal volumes of concentrate were added to each replicate to an initial density of

15-30 embryos per mL. Initial stocking density was confirmed by counting a 5 mL aliquot from at least three control replicates.

Testing was conducted at $16 \pm 2^{\circ}\text{C}$ under a 14 hour light and 10 hour dark photoperiod. Temperature, pH, dissolved oxygen, and salinity were recorded at 0 and 48 hours; temperature was also recorded at 24 hours. Total ammonia was measured in 1.2% sample at 0 and 48 hours. At the end of the exposure period, a 5 mL sub-sample was taken from each test replicate and preserved with buffered formalin. Sub-samples were counted in a Sedgwick-Rafter cell, and the total number of normal and abnormal larvae were counted.

Dissolved oxygen levels of test solutions of HSW-2 fell below 60% saturation in both the bivalve and echinoderm tests. Gentle aeration was started on Day 1, and continued for the duration of the tests. To assess the effects of aeration, control replicates 4 and 5 were aerated beginning on Day 1 for both the bivalve and echinoderm tests. No statistical differences were observed between aerated and unaerated control replicates.

2.3 STATISTICAL ANALYSIS

At the conclusion of the test, the survival data were evaluated statistically using ToxCalc™ to determine ECp, NOEC, and TU values where appropriate. ToxCalc™ is a comprehensive statistical application that follows standard guidelines for acute and chronic toxicity data analysis.

At the conclusion of the echinoderm tests, data were evaluated statistically to estimate the LC50 and IC50 values for the elutriate tests. The LC50 and IC50 values were estimated using the Probit or the Linear Interpolation (Bootstrap) Method.

The LC50 and the IC50 for the bivalve larvae copper reference toxicant test were both within two standard deviations of the laboratory means of 26.3 µg/L and 8.9 µg/L, respectively, indicating normal sensitivity of the test organisms. No laboratory means for the echinoderm larvae copper reference toxicant test have yet been established.

Statistical effects can be measured by the ECp, the estimated concentration that causes any effect, either lethal (LC) or sublethal (IC), on p% of the test population. The LCp is the point estimate of the concentration at which a lethal effect is observed in p% of the test organisms. ECp values include 95% confidence limits if available.

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The NOEC (No Observable Effect Concentration) is the highest tested concentration at which mortality is not significantly different from the control.

Water quality measurements were within the acceptable limits provided in EPA 1991. Temperature was maintained at $20 \pm 2^{\circ}\text{C}$; pH remained relatively stable, and the salinity increased slightly as would be expected in a static test. The dissolved oxygen did drop as projected at approximately 1 hour after test initiation in all of the concentration even with supplemental aeration therefore aeration was maintained in all chambers for the duration of the test. Ammonia was measured in two replicates from each concentration daily and was a potentially significant toxic component of the test for all concentrations.

3.1 *Citharichthys stigmaeus*

vc5

The LC50 for HSW-1 was 0.59%. Mortality in the effluent was rapid at the highest concentrations, occurring in 2-4 hours. There was significant mortality at 3.0, 1.5, and 0.8% concentrations compared to the control at 96 hours. The NOEC was 0.4% and the LOEC was 0.8%.

The LC50 for HSW-2 was 0.27%. Mortality in the effluent was rapid at the highest concentrations, generally occurring in 2-4 hours. There was significant mortality at 3, 1.5, 0.8 and 0.4% concentrations compared to the control at 96 hours. The NOEC was 0.2%, and the LOEC was 0.4%.

The reference toxicant test required the use of the Trimmed Spearman-Kärber method and generated an LC50 of 4.34 mg/L, an NOEC of 3.1 mg/L, and an LOEC of 6.25 mg/L. This is the first reference toxicant test on *Citharichthys* at this laboratory, therefore no database has been established by this laboratory.

3.2 *Mysidopsis bahia*

The LC50 results for both HSW effluents in the initial tests were <0.4%. Based upon the fact that no definitive LC50 could be calculated, the tests were rerun as described in the methods.

The LC50 for HSW-1 was 0.59%. Mortality in the 1.6% and 0.8% effluent was incomplete at 24 hours. At 96 hours, there was significant mortality at 1.6, 0.8, 0.4, and 0.1% concentrations compared to the control. The NOEC was 0.05% and the LOEC was 0.1%.

In the second test series the LC50 for HSW-2 was 0.12%. Mortality in the 1.6% and 0.8% effluent was complete at 24 hours. There was significant mortality at 96 hours in the 1.6, 0.8, 0.4, 0.2 and 0.1% concentrations compared to the control. The NOEC was 0.05%, and the LOEC was 0.1%.

The reference toxicant test had an LC50 of 8.90 mg/L, with an NOEC of <1.25 mg/L and an LOEC of 1.25 mg/L. This is the first reference toxicant test on *Mysidopsis* at this laboratory, therefore no database has been established.

3.3 ECHINODERM LARVAL BIOASSAY

Control survival was marginal and unacceptable according to the protocol at 64.4% with 5.7% abnormal development. Total survival was relatively high and equal to control survival in all concentrations, however all of the embryos were abnormally developed at 0.15% to 1.2% in HSW-1 and from 0.08% to 1.2% in HSW-2. The LC50 for both effluents was greater than 1.2% however the IC50 was 0.1% for HSW-1 and <0.08% for HSW-2.

The reference toxicant analysis yielded an LC50 of 11.8 µg/L and an IC50 of 10.1 µg/L. The use of the echinoderm larval bioassay is still limited and no data is available for comparison.

3.4 BIVALVE LARVAL BIOASSAY

Control survival was acceptable at 98.1% with 6.3% abnormal development. Total survival was relatively high in all concentrations, however all of the embryos were abnormally developed at 0.15% to 1.2% in HSW-1 and HSW-2. The LC50 for both effluents was greater than 1.2% however the IC50s were <0.08% for both HSW-1 and HSW-2.

The LC50 and IC50 for the bivalve larvae copper reference toxicant test were both within two standard deviations of the laboratory means of 26.3 µg/L and 8.9 µg/L, respectively, indicating normal sensitivity of the test organisms.

3.5 AMMONIA MEASUREMENTS

Ammonia in both of the HSW was very high. When measured in a 25% dilution in seawater, ammonia levels ranged from 160 to 180 mg/L. If converted to the 100% concentration, the

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ammonia level would be above 640 mg/L. Tested concentrations in the *Citharichthys* bioassay ranged from 0.08 to 0.17 mg/L in the lowest concentration (0.05%) to 3.44 to 9.65 mg/L in the 3.0% dilution. At each test concentration, HSW-2 generated the higher ammonia levels. The toxicity of ammonia to sanddabs is well documented and the measured levels in the three highest concentrations in HSW-2 and the two highest concentrations in HSW-1 were sufficient to cause toxicity in the test animals in 24 hours. The mysid test results appear to indicate a slightly higher tolerance to ammonia as has been shown in the literature.

TABLE 1

Bioassay Procedure And Organism Data
For the Survival Bioassay
Using *Citharichthys stigmaeus* (U.S. EPA 1991)

<u>Parameter</u>	<u>Data</u>
<u>Test Species</u>	<i>Citharichthys stigmaeus</i>
Supplier	J. Brezina and Associates
Collection location	Tomales Bay
Date Acquired	2/19/94
Acclimation Time	24 hours
Acclimation Water	30 ppt seawater
Acclimation Temperature	15±2°C
Age group	Juveniles, 3-5 cm TL
<u>Sample Identification</u>	
Sample ID(s)	940219-1, -2
Date Sampled	2/16/94
Date Received at ABT	2/19/94
Volume Received	Ten gallons
Sample Storage Conditions	4°C in the dark
<u>Test Procedures</u>	
Type; Duration	96 hour static acute, renewal at 48 hours
Test Dates	2/19/94 to 2/23/94
Control Water	Bodega Bay seawater
Test Temperature	15 ± 1°C
Test Photoperiod	16 L : 8 D
Initial Salinity	30 ± 2 ppt
Test Chamber	20 L polyethylene chamber
Animals/Replicate	10 animals/replicate
Exposure Volume	5 L
Replicates/Treatment	5
Feeding	None
Deviations from procedures	Due to aeration, salinity increased throughout test.

TABLE 2

Bioassay Procedure And Organism Data
For the Survival Bioassay
Using *Mysidopsis bahia* (U.S. EPA 1991)

<u>Parameter</u>	<u>Data</u>
<u>Test Species</u>	<i>Mysidopsis bahia</i>
Supplier	J. Brezina and Associates
Date Acquired	2/19/94
Acclimation Time	overnight
Acclimation Water	Shipping water
Acclimation Temperature	20 ± 2°C
Age group	larvae
<u>Sample Identification</u>	
Sample ID(s)	940219-1, -2
Date Sampled	2/16/94
Date Received at ABT	2/19/94
Volume Received	Ten gallons
Sample Storage Conditions	4°C in the dark
<u>Test Procedures</u>	
Type; Duration	Acute; static; renewal at 48 hours
Test Dates	2/19/94 to 2/23/94
Control Water	Bodega Bay seawater
Test Temperature	20 ± 2°C
Test Photoperiod	14 L : 10 D
Initial Salinity	25 ppt
Test Chamber	1000 mL jars
Animals/Replicate	10 animal/replicate
Exposure Volume	500 mL
Replicates/Treatment	5
Feeding	Brine shrimp (24 hr old nauplii)
Deviations from procedures	Due to aeration, salinity increased throughout test

TABLE 3

Bioassay Procedure And Organism Data
For the Survival Bioassay
Using *Mysidopsis bahia* (U.S. EPA 1991)

<u>Parameter</u>	<u>Data</u>
<u>Test Species</u>	<i>Mysidopsis bahia</i>
Supplier	Aquatox
Date Acquired	2/26/94
Acclimation Time	Overnight
Acclimation Water	Shipping water
Acclimation Temperature	20 ± 2°C
Age group	larvae
<u>Sample Identification</u>	
Sample ID(s)	940219-1, -2
Date Sampled	2/16/94
Date Received at ABT	2/19/94
Volume Received	Ten gallons
Sample Storage Conditions	4°C in the dark
<u>Test Procedures</u>	
Type; Duration	Acute; static; renewal at 48 hours
Test Dates	2/27/94 to 3/2/94
Control Water	Bodega Bay seawater
Test Temperature	20 ± 2°C
Test Photoperiod	14 L : 10 D
Initial Salinity	25 ppt
Test Chamber	1000 mL jars
Animals/Replicate	10 animal/replicate
Exposure Volume	500 mL
Replicates/Treatment	5
Feeding	Brine shrimp (24 hr old nauplii)
Deviations from procedures	Due to aeration, salinity increased throughout test

TABLE 4

Bioassay Procedure And Organism Data
For The Bioassay Using Larvae of
Strongylocentrotus purpuratus (modified ASTM 1994)

<u>Parameter</u>	<u>Data</u>
<u>Test Species</u>	<i>Strongylocentrotus purpuratus</i>
Supplier	A.K. Siewers, Santa Cruz, CA
Date Acquired	4/7/94
Acclimation Time	None
Acclimation Water	Not applicable
Acclimation Temperature	Not applicable
Age group	Fertilized embryos, 2 hours
<u>Sample Identification</u>	
Sample ID(s)	940404-3, -4
Date Sampled	3/30/94
Date Received at ABT	4/4/94
Volume Received	Two liters
Sample Storage Conditions	4°C in the dark
<u>Test Procedures</u>	
Type; Duration	Acute/static; 96 hours
Test Dates	4/7/94 to 4/11/94
Control Water	San Francisco Bay seawater, 0.45 µm filtered and uv-sterilized
Test Temperature	16 ± 2°C
Test Photoperiod	14 L : 10 D
Salinity	30 ± 2 ppt
Test Chamber	125 mL beakers
Animals/Replicate	Approximately 30 embryos per mL
Exposure Volume	100 mL
Replicates/Treatment	5
Feeding	None
Deviations from procedures	Chambers were gently aerated with low bubble aeration

TABLE 5

**Bioassay Procedure And Organism Data
For The 48 Hour Bioassay
Using Larvae of *Mytilus edulis* (ASTM 1993)**

<u>Parameter</u>	<u>Data</u>
<u>Test Species</u>	<i>Mytilus edulis</i>
Supplier	A.K. Siewers, Santa Cruz, CA
Date Acquired	4/7/94
Acclimation Time	None
Acclimation Water	Not applicable
Acclimation Temperature	Not applicable
Age group	Fertilized embryos, 2 hours
<u>Sample Identification</u>	
Sample ID(s)	940404-3,-4
Date Sampled	3/30/94
Date Received at ABT	4/4/94
Volume Received	Two liters
Sample Storage Conditions	4°C in the dark
<u>Test Procedures</u>	
Type; Duration	Acute; static; 48 hours
Test Dates	4/7/94 to 4/9/94
Control Water	San Francisco Bay seawater, 0.45 µm filtered and uv-sterilized
Test Temperature	16 ± 2°C
Test Photoperiod	14 L : 10 D
Salinity	30 ± 2 ppt
Test Chamber	125 mL beakers
Animals/Replicate	Approximately 30 embryos per mL
Exposure Volume	100 mL
Replicates/Treatment	3
Feeding	None
Deviations from procedures	Chambers were gently aerated with low bubble aeration

TABLE 6
SUMMARY OF RESULTS
FOR THE HIGH STRENGTH WASTE BIOASSAYS

Species	Test	Endpoint	HSW-1	HSW-2
<i>Citharichthys stigmaeus</i>	96 hr static	LC50	0.59%	0.27%
<i>Mysidopsis bahia</i>	96 hr static	LC50	0.59%	0.12%
<i>Strongylocentrotus purpuratus</i>	96 hr static	LC50	>1.2%	>1.2%
		IC50	0.10%	<0.08%
<i>Mytilus edulis</i>	48 hr static	LC50	>1.2%	>1.2%
		IC50	<0.08%	<0.08%

Note:

HSW-1: Van Camp

HSW-2: Starkist

TABLE 7

SUMMARY OF RESULTS FOR THE REFERENCE TOXICANT (S.D.S.) TEST

Citharichthys stigmaeus

Concentration (mg/L)	% Survival	ECp (mg/L)	NOEC (mg/L)	LOEC (mg/L)
Control	93.3	EC50 4.3449	3.1	6.25
1.6	80.0			
3.1	100.0			
6.2	0.0			
12.5	0.0			
25	0.0			

Mysidopsis bahia

Concentration (mg/L)	% Survival	ECp (mg/L)	NOEC (mg/L)	LOEC (mg/L)
Control	90.0	EC50 8.90 (3.04-69.22)	<1.25	1.25
1.25	70.0			
2.5	56.7			
5	46.7			
10	46.7			
20	36.7			

* Statistically significant.

ICp/LCp: Inhibition/Lethal Concentration for p% of the organisms.

NOEC: No Observable Effect Concentration.

TU: 100%/NOEC.

REFERENCES

U.S. EPA. 1991. Methods for measuring acute toxicity of effluents to freshwater and marine organisms, 4th ed. EPA 600/4-90/027, September, 1991.

ASTM. 1993. Annual Book of Standards. Vol. 11.04. Standard guide for conducting static acute toxicity tests starting with embryos of four species of saltwater bivalve mollusca. E-724-89.

ASTM. 1994. Annual Book of ASTM Standards Vol. 11.04. Guide for conducting static acute toxicity tests with echinoid embryos. Proposed Standard in review.

A
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ANALYTICAL DATA

A

APPENDIX TABLE 1

SAMPLE WATER QUALITY

Date	Day	Sample	pH (units)	DO (mg/L)	Total NH3 (mg/L)	Initial Salinity (ppt)
4/7/94	0	HSW-1, 1.2%	7.62	8.0	62.5	26
	0	HSW-2, 1.2%	6.87	7.9	51.6	26
4/9/94	2	HSW-1, 1.2%	-	-	26.4	-
	2	HSW-2, 1.2%	-	-	41.2	-
4/11/94	4	HSW-1, 1.2%	-	-	33.5	-
	4	HSW-2, 1.2%	-	-	41.9	-

APPENDIX TABLE 2

Citharichthys stigmatæus
WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST
HSW-1

Concentration (%)	Rep	Day 0					Day 1					Day 2					Day 3					Day 4				
		pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal
Control	1	8.02	6.2	0.02	14.0	32.0	8.07	5.5	0.01	13.2	31.5	8.08	5.5		13.8	32.9	8.03	6.0		14.0	35.0	8.06	6.1	0.02	14.4	36.0
	2						8.11	5.8		13.7	31.0	8.13	5.6	0.12	14.2	31.7	8.12	6.0		14.3	33.0	8.13	6.1		15.0	33.0
	3						8.10	6.0		13.8	30.9	8.12	5.7		14.2	31.8	8.11	6.0		14.4	32.0	8.12	5.8		15.2	33.0
	4						8.10	6.0		13.2	31.6	8.13	5.7		13.6	33.1	8.11	6.0	<0.10	13.9	35.0	8.13	5.6		14.6	36.0
	5						8.10	6.0		13.3	31.7	8.12	5.6		13.9	33.3	8.12	6.0		14.0	34.0	8.13	5.8		14.7	37.0
0.05	1	8.00	6.3	0.19	14.0	32.2	8.04	6.0	0.08	13.5	33.8	8.07	5.6		13.9	36.2	8.07	6.0		14.0	38.0	8.07	5.8	0.10	14.8	40.0
	2						8.03	6.0		13.6	33.8	8.07	5.5	0.05	13.9	36.4	8.04	6.0		14.1	38.0	8.06	5.6		14.7	40.0
	3						8.05	6.0		13.5	32.7	8.10	5.5		14.1	33.6	8.08	6.0		14.2	35.0	8.10	5.6		14.6	35.0
	4						8.01	6.0		13.5	32.3	8.07	5.6		14.1	33.4	8.06	6.0	<0.10	14.2	34.0	8.04	5.8		14.7	35.0
	5						8.05	5.9		13.6	33.1	8.09	5.6		14.1	34.1	8.09	6.0		14.2	35.0	8.10	5.8		14.9	36.0
0.1	1	8.01	6.2	0.25	14.0	32.1	8.06	6.0	0.13	13.5	31.8	8.12	5.6		13.9	32.6	8.11	6.0		14.1	34.0	8.13	5.8	0.12	14.9	34.0
	2						8.03	5.9		13.8	31.7	8.10	5.7	0.08	14.2	32.6	8.10	6.0		14.4	33.0	8.10	5.8		14.9	34.0
	3						8.01	5.8		13.3	32.8	8.08	5.7		13.8	34.8	8.06	5.9		14.0	37.0	8.06	5.6		14.4	39.0
	4						8.04	5.9		13.8	32.6	8.12	5.8		14.5	33.9	8.11	6.0	<0.10	14.6	35.0	8.11	5.7		14.9	36.0
0.2	1	8.01	6.0	0.54	14.0	32.1	8.04	5.7	0.20	14.2	30.0	8.14	5.9		14.4	31.1	8.13	6.0		14.3	32.0	8.13	6.0	0.17	14.9	34.0
	2						8.01	5.8		14.1	29.9	8.14	5.8	0.17	14.5	30.5	8.16	6.0		14.6	31.0	8.16	5.9		14.9	32.0
	3						7.98	5.8		13.9	29.8	8.12	5.8		14.2	30.3	8.13	5.9		14.9	31.0	8.14	5.9		15.0	32.0
	4						8.02	5.8		13.9	29.8	8.15	5.8		14.2	30.5	8.15	6.3	NT	14.9	31.0	8.16	5.8		15.0	32.0
	5						8.03	5.8		13.8	29.8	8.13	5.8		14.2	30.5	8.15	6.3		14.9	31.0	8.17	5.8		15.0	32.0
0.4	1	7.93	6.1	0.89	14.0	32.0	7.95	5.4	0.33	13.7	30.1	8.12	5.4		14.2	30.8	8.14	6.3		14.3	32.0	8.17	5.8	0.31	15.0	32.0
	2						7.98	5.6		14.4	30.2	8.13	5.8	0.25	14.8	31.1	8.17	6.3		14.9	32.0	8.18	5.8		14.7	33.0
	3						8.00	5.9		14.4	30.2	8.15	5.7		14.3	31.6	8.18	6.3		14.6	33.0	8.06	5.8		14.6	34.0
	4						7.76	4.6		14.0	29.9	8.06	5.8		14.5	30.3	8.09	6.2	0.17	14.7	31.0	8.11	5.8		14.6	36.0
	5						7.93	5.2		13.5	30.4	8.11	5.6		14.0	31.4	8.13	6.2		14.0	32.0	8.19	5.6		14.3	34.0
0.8	1	7.68	6.1	2.01	14.0	32.0	7.89	5.2	0.64	13.7	30.8	8.15	5.6		14.1	31.7	8.15	6.2		14.2	33.0	8.10	5.8	0.51	14.7	33.0
	3						7.82	5.1		13.1	31.2	8.09	5.6	0.40	13.7	32.6	8.06	6.3		13.90	34.0	8.10	5.8		14.20	36.0
	4						7.95	5.4		14.1	30.8	8.16	5.5		14.5	32.0	8.17	6.4	0.48	14.3	34.0	8.18	5.8		14.4	35.0
	5						7.88	5.4		13.2	31.5	8.13	5.7		14.5	32.7	8.16	6.3		14.5	34.0	8.21	5.8		14.3	35.0
1.5	1	7.51	6.0	3.56	14.0	32.2	7.83	5.2	1.43	13.3	32.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.76	4.8		13.5	31.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.75	5.0		12.9	32.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.76	5.2		12.9	32.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.76	5.1		12.9	32.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3.0	1	7.23	5.9	11.1	14.0	32.1	7.85	5.6	3.44	13.6	33.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.74	4.6		13.9	33.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.81	5.0		13.9	33.8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.75	4.7		14.1	33.6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.81	5.0		19.2	33.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Min		7.23	5.9	0.02	14.0	32.0	7.74	4.6	0.01	12.9	29.8	8.06	5.4	0.05	13.6	30.3	8.03	5.9	<0.10	13.9	31.0	8.04	5.6	0.02	14.2	32.0
Max		8.02	6.3	11.1	14.0	32.2	8.11	6.0	3.44	19.2	33.8	8.16	5.9	0.40	14.8	36.4	8.18	6.4	0.48	14.9	38.0	8.21	6.1	0.51	15.2	40.0

Note: — = All animals dead.

NT = Not taken.

0.1 replicate 5 not stocked.

0.8 replicate 2 lost due to lab error.

APPENDIX TABLE 2 (Cont'd)

Citharichthys stigmaeus
WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST
HSW-2

Concentration (%)	Rep	Day 0					Day 1					Day 2					Day 3					Day 4				
		pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal
Control	1	8.02	6.2	0.02	14.0	32.0	8.08	5.5	0.01	13.2	31.5	8.02	5.5	13.8	32.9	8.03	6.0		14.0	35.0	8.06	6.1	0.02	14.4	36.0	
	2						8.11	5.8		13.7	31.0	8.13	5.6	0.12	14.2	31.7	8.12	6.0		14.3	33.0	8.13	6.1		15.0	33.0
	3						8.10	6.0		13.8	30.9	8.12	5.7		14.2	31.8	8.11	6.0		14.4	32.0	8.12	5.8		15.2	33.0
	4						8.10	6.0		13.2	31.6	8.13	5.7		13.6	33.1	8.11	6.0	<0.10	13.9	35.0	8.13	5.6		14.6	36.0
	5						8.10	6.0		13.3	31.7	8.12	5.6		13.9	33.3	8.12	6.0		14.0	34.0	8.13	5.8		14.7	37.0
0.05	1	7.89	6.1	0.32	14.0	32.0	7.98	6.0		13.5	36.2	8.02	5.6	13.9	41.1	8.02	6.4		14.0	38.0	8.03	5.2	0.13	14.4	40.0	
	2						8.03	6.2	0.17	14.5	34.0	8.11	5.6	0.12	15.0	35.4	8.13	6.4		15.2	38.0	8.15	5.6		15.2	40.0
	3						8.01	6.0		13.6	33.7	8.05	5.7		14.1	34.9	8.10	6.3		14.4	36.0	8.10	5.6		14.2	37.0
	4						8.02	6.0		13.3	34.5	8.04	5.8		13.7	36.9	8.07	6.3	<0.10	13.9	38.0	8.06	5.6		14.0	40.0
	5						8.01	6.0		13.3	34.5	8.04	5.6		13.8	36.5	8.05	6.3		14.0	38.0	8.06	5.6		14.0	40.0
0.1	1	7.96	6.0	0.56	14.0	32.2	8.02	6.1		13.3	35.0	8.03	5.4	13.7	37.8	8.04	6.2		13.9	40.0	8.06	5.8	0.12	13.9	40.0	
	2						8.03	6.1	0.24	14.2	33.6	8.09	5.5	0.13	14.9	34.5	8.11	6.3		14.9	35.0	8.13	5.8		14.6	36.0
	3						8.02	6.0		13.8	34.2	8.05	5.7		14.2	36.1	8.06	6.3		14.4	38.0	8.08	5.8		14.3	40.0
	4						8.02	5.9		14.3	33.5	8.07	5.5		14.9	34.2	8.09	6.3	<0.10	15.0	35.0	8.11	5.8		14.7	36.0
	5						8.04	6.1		13.2	33.6	8.07	5.6		14.8	34.4	8.11	6.3		14.0	35.0	8.13	5.8		13.9	36.0
0.2	1	7.87	6.1	1.32	14.0	32.0	8.03	6.0		13.2	33.5	8.11	5.6	13.9	34.3	8.12	6.3		14.1	35.0	8.15	5.8	0.20	13.8	36.0	
	2						8.02	6.0	0.53	13.2	33.6	8.10	5.7	0.20	13.9	34.6	8.12	6.3		14.1	35.0	8.14	5.8		13.7	37.0
	3						8.03	6.0		13.5	33.5	8.10	5.8		14.1	34.1	8.13	6.3		14.3	35.0	8.15	5.8		13.9	36.0
	4						8.01	6.0		13.5	33.7	8.09	5.8		14.0	34.8	8.12	6.3	0.22	14.3	36.0	8.14	5.8		13.9	37.0
	5						8.02	6.0		13.8	33.8	8.10	5.7		14.2	34.8	8.04	6.3		14.3	35.0	8.15	5.8		14.2	36.0
0.4	1	7.66	6.0	3.00	14.0	32.1	7.95	5.8		13.2	35.1	7.99	5.4	13.8	38.2	8.08	6.3		13.9	41.0	8.05	5.8	0.30	13.7	40.0	
	2						7.97	5.8	0.86	13.2	34.5	8.06	5.3	0.32	13.9	36.3	8.10	6.3		14.1	38.0	8.08	5.8		13.7	41.0
	3						7.99	6.0		14.5	33.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.99	5.9		14.4	33.5	7.89	5.1		15.0	34.1	—	—	—	—	—	—	—	—	—	—
	5						7.99	5.9		14.4	33.6	8.04	5.4		14.8	34.5	8.13	6.3	0.23	14.9	35.0	8.15	5.8		15.2	36.0
0.8	1	7.35	6.0	6.34	14.0	32.0	7.88	5.4		13.5	35.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.93	5.7	1.95	14.1	33.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.91	5.7		13.9	33.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.93	5.7		13.9	33.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.92	5.8		14.2	33.9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1.5	2	7.00	5.9	14.6	14.0	32.0	7.84	5.5		14.1	33.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.80	5.4	4.23	14.2	33.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.85	5.4		13.9	33.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.85	5.4		13.9	33.4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3.0	1	6.81	5.7	28.5	14.0	32.0	7.89	5.7		13.9	33.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.86	5.9	9.65	13.8	33.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.88	5.9		13.6	33.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.81	5.8		13.0	34.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.81	5.8		12.9	34.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Min		6.81	5.7	0.02	14.0	32.0	7.80	5.4	0.17	12.9	30.9	7.89	5.1	0.12	13.6	31.7	8.02	6.0	<0.10	13.9	32.0	8.03	5.2	0.12	13.7	33.0
Max		8.02	6.2	28.50	14.0	32.2	8.11	6.2	9.65	14.5	36.2	8.13	5.8	0.32	15.0	41.1	8.13	6.4	0.23	15.2	41.0	8.15	6.1	0.30	15.2	41.0

Note: — = All animals dead.

APPENDIX TABLE 3

Citharichthys stigmaeus
SURVIVAL DATA FOR EFFLUENT TEST
HSW-1

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
0.05	1	10	10	10	10	10	100	98.0
	2	10	10	9	9	9	90	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
0.1	1	10	10	10	10	10	100	97.5
	2	10	10	10	10	10	100	
	3	10	10	10	10	9	90	
	4	10	10	10	10	10	100	
0.2	1	10	10	10	10	10	100	98.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	9	9	90	
0.4	1	10	10	10	10	10	100	84.0
	2	10	7	6	6	6	60	
	3	10	10	8	8	8	80	
	4	10	9	9	9	9	90	
	5	10	10	9	9	9	90	
0.8	1	10	5	3	3	1	10	32.5
	3	10	10	9	9	9	90	
	4	10	9	1	1	0	0	
	5	10	5	5	3	3	30	
1.5	1	10	0	—	—	—	0	0.0
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	
30	1	10	0	—	—	—	0	0.0
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	

Notes: — = All animals dead.

APPENDIX TABLE 3 (Cont'd)

Citharichthys stigmaeus
SURVIVAL DATA FOR EFFLUENT TEST
HSW-2

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
0.05	1	10	10	10	10	9	90	94.0
	2	10	10	10	10	9	90	
	3	10	10	10	10	10	100	
	4	10	10	10	10	9	90	
	5	10	10	10	10	10	100	
0.1	1	10	10	10	9	9	90	98.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
0.2	1	10	10	10	10	10	100	96.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	9	9	9	90	
	5	10	10	9	9	9	90	
0.4	1	10	4	3	2	2	20	14.0
	2	10	4	3	3	2	20	
	3	10	0	—	—	—	0	
	4	10	3	0	—	—	0	
	5	10	3	3	3	3	30	
0.8	1	10	0	—	—	—	0	0.0
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	
1.5	2	10	0	—	—	—	0	0.0
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	
3	1	10	0	—	—	—	0	0.0
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	

Notes: — = All animals dead.

APPENDIX TABLE 4

Citharichthys stigmaeus WATER QUALITY MEASUREMENTS FOR REFERENCE TOXICANT (S.D.S) TEST

Concentration (mg/L)	Rep	Day 0				Day 1			
		pH	DO	°C	Sal	pH	DO	°C	Sal
Control	1	8.02	5.8	15.9	32	7.20	5.7	15.2	31
	2					7.31	5.0	15.1	31
	3					7.31	4.7	15.1	31
1.6	1	8.03	5.8	15.9	32	7.49	4.7	15.1	31
	2					7.52	4.2	15.1	31
	3					7.51	4.1	15.2	31
3.1	1	8.03	5.8	15.9	32	7.49	4.0	15.1	31
	2					7.43	4.0	15.2	30
	3					7.51	3.9	15.1	31
6.25	1	8.03	5.8	15.9	32	7.49	4.1	15.1	31
	2					7.48	4.1	15.1	30
	3					7.47	4.0	15.1	31
12.5	1	8.04	5.8	15.9	32	7.40	3.9	15.1	31
	2					7.44	3.7	15.1	31
	3					7.51	3.7	15.1	31
25	1	8.03	5.7	15.9	32	7.44	3.0	15.1	31
	2					7.42	3.1	15.1	31
	3					7.36	3.2	15.0	31
Min		8.02	5.7	15.9	32	7.20	3.0	15.0	30
Max		8.04	5.8	15.9	32	7.52	5.7	15.2	31

APPENDIX TABLE 5

Citharichthys stigmaeus SURVIVAL DATA FOR REFERENCE TOXICANT (S.D.S.) TEST

Concentration (mg/L)	Rep	Initial Added	Day 1	% Survival	Average % Survival
Control	1	5	4	80	93.3
	2	5	5	100	
	3	5	5	100	
1.6	1	5	2	40	80.0
	2	5	5	100	
	3	5	5	100	
3.1	1	5	5	100	100.0
	2	5	5	100	
	3	5	5	100	
6.25	1	5	0	0	0.0
	2	5	0	0	
	3	5	0	0	
12.5	1	5	0	0	0.0
	2	5	0	0	
	3	5	0	0	
25	1	5	0	0	0.0
	2	5	0	0	
	3	5	0	0	

APPENDIX TABLE 6

Mysidopsis bahia
WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST
HSW-1

Concentration (%)	Rep	Day 0					Day 1					Day 2					Day 3					Day 4				
		pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal
Control	1	8.06	5.4		18.0	32.0	8.14	5.2	<0.01	19.6	32.0	8.11	5.1		19.8	33.0	8.11	4.6	<0.10	21.7	33.9	8.08	4.9	<0.10	21.1	34.1
	2						8.13	5.2		19.9	32.0	8.08	5.2		20.1	33.0	8.07	4.6		21.6	33.6	8.07	5.1		21.1	34.1
	3						8.16	5.1		19.7	32.0	8.12	5.4		20.2	33.6	8.11	4.5		21.6	34.7	8.09	5.1		21.1	34.0
	4						8.16	5.2		19.7	32.0	8.12	5.4		20.2	33.3	8.14	4.5		21.6	33.9	8.12	5.0		21.0	33.8
	5						8.15	5.2		19.7	32.0	8.11	5.5		20.2	33.1	8.11	4.5		21.5	34.0	8.10	4.9		21.0	34.1
0.05	1	8.08	5.4	0.13	18.0	32.0	8.14	5.2	0.12	19.8	32.0	8.13	5.4	0.14	20.1	33.6	8.13	4.5	0.13	21.7	34.8	8.12	5.0	0.13	20.9	34.1
	2						8.15	5.2		19.8	32.0	8.14	5.6		20.2	32.7	8.15	4.4		21.6	33.6	8.13	5.0		21.1	34.1
	3						8.13	5.2		19.6	32.0	8.11	5.6		20.2	32.8	8.13	4.5		21.6	33.6	8.14	5.1		21.1	34.3
	4						8.10	5.0		19.6	32.0	8.11	5.6		20.1	32.3	8.12	4.5		21.4	32.8	8.12	5.1		20.0	34.2
	5						8.04	5.1		19.5	32.0	8.08	5.5		20.1	32.4	8.06	4.5		21.3	33.3	8.10	5.0		20.0	34.0
0.1	1	8.06	5.4	0.25	18.0	32.0	8.02	5.0	0.19	19.6	32.0	8.09	5.4	0.29	20.2	33.1	8.06	4.6	0.23	21.7	33.9	8.12	5.0	0.24	21.0	35.1
	2						7.92	5.0		19.6	32.0	8.03	5.4		20.1	33.1	8.02	4.4		21.5	34.1	8.10	5.1		21.0	35.0
	3						7.99	4.9		19.5	32.0	8.10	5.3		19.9	33.0	8.13	4.4		21.3	35.0	8.13	4.9		20.9	35.1
	4						8.00	5.0		19.4	32.0	8.10	5.3		19.9	33.3	8.10	4.5		21.2	34.7	8.10	5.0		20.9	35.1
	5						8.02	5.0		19.3	32.0	8.10	5.3		19.9	33.5	8.16	4.6		21.1	35.4	8.09	5.0		20.9	35.7
0.2	1	8.04	5.2	0.61	18.0	32.0	7.91	5.0	0.38	19.6	32.0	8.11	5.4	0.38	20.0	32.6	8.14	4.8	0.41	21.5	34.2	8.18	4.9	0.52	21.0	34.8
	2						7.75	4.4		19.1	32.0	8.07	5.4		19.6	36.0	8.05	4.6		20.9	41.1	8.21	5.0		21.0	41.2
	3						7.58	3.8		19.0	32.0	8.04	5.5		19.5	35.2	8.04	4.5		20.7	38.7	8.20	5.0		21.1	38.7
	4						7.76	4.2		18.9	32.0	8.06	5.5		19.6	35.6	8.05	4.5		20.9	38.3	8.17	5.1		21.0	38.9
	5						7.81	4.4		19.0	32.0	8.07	5.4		19.5	35.0	8.11	4.5		20.9	35.9	8.17	5.1		21.0	36.2
0.4	1	8.02	5.2	1.17	18.0	32.0	7.83	4.2	0.71	19.5	32.0	8.16	5.4	0.74	19.9	32.9	8.20	4.6	0.82	21.4	34.0	8.21	5.1	1.09	20.9	34.8
	2						7.87	4.6		19.5	32.0	8.18	5.4		19.9	32.9	8.20	4.6		21.0	33.7	8.18	5.2		20.9	34.0
	3						7.73	3.8		19.5	32.0	8.19	5.2		19.9	33.0	8.20	4.6		21.2	33.8	8.19	5.1		20.9	33.9
	4						7.79	4.8		19.4	32.0	8.17	5.1		19.9	32.9	8.15	4.5		21.2	33.5	8.21	5.1		20.8	33.9
	5						7.91	4.4		19.4	32.0	8.19	5.1		19.9	33.0	8.20	4.5		21.0	33.6	8.21	5.1		20.8	33.9
0.8	1	7.92	5.3	3.62	19.9	32.0	7.62	3.8	1.52	19.5	32.0	8.22	5.3	1.38	19.9	33.2	8.23	4.6	1.42	21.3	33.9	8.22	5.1	1.53	21.0	34.1
	2						7.70	3.4		19.5	32.0	8.21	5.2		19.9	32.4	8.21	4.5		21.2	33.5	8.22	5.0		21.1	34.2
	3						7.61	3.4		19.4	32.0	8.19	5.1		19.9	33.2	8.19	4.4		21.1	34.0	8.21	5.0		21.0	34.7
	4						7.82	3.8		19.4	32.0	8.22	5.0		19.9	32.9	8.23	4.4		21.2	34.0	8.27	5.1		21.0	34.7
	5						7.59	3.0		19.4	32.0	8.24	5.0		19.9	33.0	8.23	4.4		21.2	34.0	8.24	5.0		21.0	34.2
1.6	1	7.88	5.2	7.14	20.2	32.0	7.61	1.4	3.27	19.6	32.0	8.25	5.2	3.45	20.1	32.7	8.23	4.6	3.27	21.3	33.8	8.28	4.9	3.12	21.1	34.1
	2						7.67	1.8		19.4	32.0	8.25	5.1		19.9	32.9	8.22	4.5		21.1	33.7	8.24	4.9		21.1	34.2
	3						7.68	1.8		18.6	32.0	8.15	5.0		19.5	34.4	—	—	—	—	—	—	—	—	—	—
	4						7.51	0.4		19.1	32.0	8.24	5.0		19.6	32.4	—	—	—	—	—	—	—	—	—	—
	5						7.70	2.4		18.9	32.0	8.19	5.0		19.4	36.1	8.12	4.5		20.6	40.8	8.31	5.0		20.9	33.9
Min		7.88	5.2	0.13	18.0	32.0	7.51	0.4	<0.01	18.6	32.0	8.03	5.0	0.14	19.4	32.3	8.02	4.4	<0.10	20.6	32.8	8.07	4.9	<0.10	20.0	33.8
Max		8.08	5.4	7.14	20.2	32.0	8.16	5.2	3.27	19.9	32.0	8.25	5.6	3.45	20.2	36.1	8.23	4.8	3.27	21.7	41.1	8.31	5.2	3.12	21.1	41.2

Note: — = All animals dead.

APPENDIX TABLE 6 (Cont'd)

Mysidopsis bahia
WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST
HSW-2

Concentration (%)	Rep	Day 0					Day 1					Day 2					Day 3					Day 4				
		pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal
Control	1	8.06	5.4		18.0	32.0	8.14	5.2	<0.01	19.6	32.0	8.11	5.1		19.8	33.0	8.11	4.6	<0.10	21.7	33.9	8.08	4.9	<0.10	21.1	34.1
	2						8.13	5.2		19.9	32.0	8.08	5.2		20.1	33.0	8.07	4.6		21.6	33.6	8.07	5.1		21.1	34.1
	3						8.16	5.1		19.7	32.0	8.12	5.4		20.2	33.6	8.11	4.5		21.6	34.7	8.09	5.1		21.1	34.0
	4						8.16	5.2		19.7	32.0	8.12	5.4		20.2	33.3	8.14	4.5		21.6	33.9	8.12	5.0		21.0	33.8
	5						8.15	5.2		19.7	32.0	8.11	5.5		20.2	33.1	8.11	4.5		21.5	34.0	8.10	4.9		21.0	34.1
0.05	1	8.04	5.2	0.13	19.9	32.0	8.00	5.0	0.11	19.2	32.0	8.11	4.9	0.12	19.9	32.7	8.12	4.6	0.12	21.1	33.6	8.18	5.0	0.11	21.0	34.1
	2						7.97	4.8		19.1	32.0	8.09	4.9		19.6	33.0	8.08	4.5		20.9	33.7	8.19	5.1		21.1	34.2
	3						7.96	4.8		18.9	32.0	8.07	4.8		19.4	34.0	8.06	4.4		20.6	34.7	8.22	5.1		21.1	34.1
	4						7.96	4.8		18.6	32.0	8.08	4.8		19.2	34.2	8.05	4.4		20.4	35.8	8.21	5.1		21.1	34.1
	5						8.03	4.9		18.6	32.0	8.09	4.8		19.3	34.4	8.04	4.5		20.4	36.6	8.19	5.0		21.0	34.2
0.1	1	8.05	5.2	0.25	19.6	32.0	8.00	5.0	0.18	19.1	32.0	8.12	4.9	0.16	19.6	34.7	8.15	4.4	0.17	20.9	36.0	8.19	5.0	0.17	21.0	36.3
	2						7.97	5.0		19.1	32.0	8.15	5.0		19.6	34.6	8.15	4.5		20.7	33.7	8.20	5.0		21.1	36.4
	3						8.01	5.0		18.9	32.0	8.15	4.9		19.4	35.4	8.15	4.6		20.5	34.7	8.16	5.0		21.1	34.7
	4						7.97	4.9		18.8	32.0	8.15	4.9		19.4	35.2	8.14	4.4		20.3	36.0	8.17	5.0		21.1	35.2
	5						8.07	4.9		18.7	32.0	8.17	5.0		19.3	34.6	8.18	4.4		20.2	39.0	8.19	5.0		21.1	39.7
0.2	1	7.96	5.2	0.61	20.1	32.0	7.74	4.4	0.57	19.0	32.0	8.16	5.0	0.30	18.7	31.8	8.14	4.4	0.32	19.4	32.5	8.21	5.0	0.39	21.1	33.4
	2						7.78	4.6		19.1	32.0	8.15	4.9		18.7	32.5	8.13	4.5		19.4	32.8	8.09	5.1		21.0	34.2
	3						7.81	4.5		18.9	32.0	8.14	5.0		18.6	32.4	8.15	4.4		19.2	32.9	8.21	4.9		21.0	34.1
	4						7.85	4.6		18.8	32.0	8.16	5.0		18.4	32.4	8.16	4.4		19.1	33.7	8.23	4.9		21.0	34.1
	5						7.81	4.6		18.6	32.0	8.15	5.0		18.4	33.7	8.15	4.5		19.1	35.1	8.16	5.1		21.1	34.2
0.4	1	7.92	5.2	1.17	20.2	32.0	7.76	3.6	1.08	19.1	31.0	8.15	5.0	1.10	18.9	31.5	8.19	4.6	1.20	19.5	32.4	8.23	5.1	1.16	21.1	33.7
	2						7.75	3.6		19.1	32.0	8.16	5.0		18.6	33.9	8.14	4.5		19.5	35.9	8.18	5.1		21.1	36.2
	3						7.59	1.8		18.7	32.0	8.14	5.0		18.4	34.1	8.10	4.4		19.2	36.5	8.19	5.1		21.1	37.0
	4						7.73	3.4		18.6	32.0	8.16	5.0		18.4	33.7	8.14	4.3		19.2	35.1	8.19	5.0		21.1	36.1
	5						7.80	3.6		18.6	32.0	8.16	5.0		18.5	33.8	8.16	4.3		19.2	35.6	8.22	5.0		21.1	36.1
0.8	1	7.79	5.2	3.62	20.2	32.0	7.52	1.2	2.17	19.0	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.61	1.8		19.0	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.54	2.2		18.9	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.71	2.2		18.9	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.66	2.6		18.9	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1.6	1	7.67	5.0	7.14	20.0	32.0	7.58	2.8	4.43	19.0	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.39	2.6		18.9	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.46	1.4		18.9	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.38	1.6		18.9	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.49	1.6		18.9	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Min		7.67	5.0	0.13	18.0	32.0	7.38	1.2	<0.01	18.6	31.0	8.07	4.8	0.12	18.4	31.5	8.04	4.3	<0.10	19.1	32.4	8.07	4.9	<0.10	21.0	33.4
Max		8.06	5.4	7.14	20.2	32.0	8.16	5.2	4.43	19.9	32.0	8.17	5.5	1.10	20.2	35.4	8.19	4.6	1.20	21.7	39.0	8.23	5.1	1.16	21.1	39.7

Note: — = All animals dead.

APPENDIX TABLE 7

Mysidopsis bahia
SURVIVAL DATA FOR EFFLUENT TEST
HSW-1

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	9	10	100	92.0
	2	10	10	10	10	9	90	
	3	10	10	10	9	9	90	
	4	10	10	10	10	9	90	
	5	10	10	10	10	9	90	
0.05	1	10	9	9	9	9	90	78.0
	2	10	10	10	9	8	80	
	3	10	10	8	8	7	70	
	4	10	9	7	7	6	60	
	5	10	10	9	8	9	90	
0.1	1	10	6	5	2	6	60	68.0
	2	10	10	9	5	8	80	
	3	10	8	8	7	6	60	
	4	10	8	6	7	8	80	
	5	10	9	8	8	6	60	
0.2	1	10	9	8	4	7	70	76.0
	2	10	8	7	5	7	70	
	3	10	9	7	7	8	80	
	4	10	9	8	7	8	80	
	5	10	10	9	8	8	80	
0.4	1	10	8	7	5	6	60	66.0
	2	10	8	7	6	6	60	
	3	10	8	8	6	6	60	
	4	10	8	7	7	8	80	
	5	10	10	9	8	7	70	
0.8	1	10	5	*	*	3	30	24.0
	2	10	4	*	*	3	30	
	3	10	6	*	*	3	30	
	4	10	4	*	*	3	30	
	5	10	3	*	*	0	0	
1.6	1	10	3	*	*	0	0	0.0
	2	10	2	*	*	0	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	1	*	*	0	0	

Notes: — = All animals dead.
* Sample too turbid to do counts.

APPENDIX TABLE 7 (Cont'd)

Mysidopsis bahia
SURVIVAL DATA FOR EFFLUENT TEST
HSW-2

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	9	10	100	92.0
	2	10	10	10	10	9	90	
	3	10	10	10	9	9	90	
	4	10	10	10	10	9	90	
	5	10	10	10	10	9	90	
0.05	1	10	10	10	10	9	90	66.0
	2	10	9	9	8	6	60	
	3	10	10	9	8	7	70	
	4	10	8	8	8	5	50	
	5	10	9	8	8	6	60	
0.1	1	10	7	7	7	6	60	48.0
	2	10	8	7	5	4	40	
	3	10	7	6	4	7	70	
	4	10	8	7	4	4	40	
	5	10	7	7	6	3	30	
0.2	1	10	6	4	2	2	20	38.0
	2	10	5	5	4	2	20	
	3	10	6	6	3	5	50	
	4	10	6	6	4	6	60	
	5	10	5	4	2	4	40	
0.4	1	10	5	*	*	1	10	8.0
	2	10	3	*	*	2	20	
	3	10	4	*	*	1	10	
	4	10	3	*	*	0	0	
	5	10	3	*	*	0	0	
0.8	1	10	0	—	—	—	0	0.0
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	
1.6	1	10	0	—	—	—	0	0.0
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	

Notes: — = All animals dead.
* Sample too turbid to do counts.

APPENDIX TABLE 8

Mysidopsis bahia
WATER QUALITY MEASUREMENTS
FOR REFERENCE TOXICANT (S.D.S) TEST

Concentration (mg/L)	Rep	Day 0				Day 1				Day 2				Day 3				Day 4			
		pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal
Control	1	8.03	5.6	20.9	32.0	8.00	4.8	21.2	32.0	7.67	5.4	21.6	33.0	7.90	3.8	21.6	33.9	7.93	4.1	21.1	34.0
	2					8.02	4.8	21.2	32.0	7.72	5.4	21.5	33.0	7.91	3.7	21.6	30.9	7.94	4.0	21.1	34.1
	3					8.03	4.8	21.3	32.0	7.70	5.3	21.6	33.0	7.90	3.8	21.8	33.8	7.94	4.0	21.1	34.2
1.25	1	8.04	5.4	20.9	32.0	8.00	4.8	21.3	32.0	7.58	5.2	21.6	33.0	7.90	3.6	21.8	33.8	7.94	4.0	20.9	34.1
	2					8.02	4.8	21.2	32.0	7.54	5.1	21.6	33.0	7.93	3.5	21.8	33.7	7.93	4.0	21.0	34.3
	3					8.03	4.8	21.2	32.0	7.38	5.1	21.6	33.0	7.95	3.5	21.7	33.8	7.95	3.9	21.0	34.7
2.5	1	8.04	5.4	20.9	32.0	8.01	4.8	21.3	32.0	7.62	5.1	21.6	33.0	7.96	3.6	21.8	33.8	7.99	3.9	20.9	34.1
	2					8.02	4.8	21.1	32.0	7.42	5.1	21.6	33.0	7.93	3.6	21.8	33.6	7.92	3.8	20.9	34.0
	3					8.02	4.6	21.1	32.0	7.47	5.0	21.6	33.0	7.93	3.6	21.7	33.9	7.91	3.8	21.0	33.9
5	1	8.04	5.4	21.1	32.0	8.00	4.8	21.1	32.0	7.32	4.7	21.6	33.0	7.98	3.7	21.8	33.1	7.92	3.8	21.0	33.8
	2					8.00	4.7	21.1	32.0	7.38	4.8	21.6	33.0	7.92	3.5	21.8	33.0	7.92	3.9	21.0	33.7
	3					7.98	4.7	21.1	32.0	7.31	4.6	21.5	33.0	7.92	3.5	21.8	33.9	7.91	3.9	21.0	33.9
10	1	8.03	5.4	21.2	32.0	7.91	4.6	21.2	32.0	7.30	4.1	21.5	33.0	7.86	3.6	21.9	33.7	7.89	3.9	20.9	34.0
	2					7.91	4.5	21.2	32.0	7.31	4.2	21.5	33.0	7.88	3.6	21.9	33.8	7.89	3.9	20.9	33.9
	3					7.91	4.3	21.2	32.0	7.31	4.2	21.6	33.0	7.87	3.6	22.0	33.6	7.91	3.9	21.0	34.1
20	1	8.02	5.3	20.8	32.0	7.85	4.4	20.9	32.0	7.20	4.0	21.6	33.0	7.78	3.7	21.8	33.4	7.90	3.9	21.0	33.9
	2					7.85	4.4	20.9	32.0	7.21	4.0	21.6	33.0	7.75	3.8	21.8	33.4	7.88	3.8	21.0	33.4
	3					7.86	4.2	20.9	32.0	7.21	4.0	21.5	33.0	7.78	3.8	21.8	33.2	7.88	3.9	21.0	33.9
Min		8.02	5.3	20.8	32.0	7.85	4.2	20.9	32.0	7.20	4.0	21.5	33.0	7.75	3.5	21.6	30.9	7.88	3.8	20.9	33.4
Max		8.04	5.6	21.2	32.0	8.03	4.8	21.3	32.0	7.72	5.4	21.6	33.0	7.98	3.8	22.0	33.9	7.99	4.1	21.1	34.7

APPENDIX TABLE 9

Mysidopsis bahia

SURVIVAL DATA FOR REFERENCE TOXICANT (S.D.S.) TEST

Concentration (mg/L)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	9	9	90	90.0
	2	10	10	10	10	9	90	
	3	10	10	10	9	9	90	
1.25	1	10	9	9	8	7	70	70.0
	2	10	10	9	6	6	60	
	3	10	9	8	8	8	80	
2.5	1	10	9	8	6	5	50	56.7
	2	10	10	8	6	6	60	
	3	10	10	8	6	6	60	
5	1	10	11	9	5	5	50	46.7
	2	10	9	7	5	4	40	
	3	10	10	9	7	5	50	
10	1	10	10	9	7	5	50	46.7
	2	10	9	9	4	4	40	
	3	10	9	7	5	5	50	
20	1	10	7	5	3	2	20	36.7
	2	10	10	8	7	5	50	
	3	10	10	8	5	4	40	

APPENDIX TABLE 10

Strongylocentrotus purpuratus
WATER QUALITY MEASUREMENTS FOR THE EFFLUENT TEST
Test Dates: 4/7-4/11/94

Site	Concentration (%)	°C	Day 0			°C	Day 1			°C	Day 2			°C	Day 3			°C	Day 4		
			DO	pH	Sal		DO	pH	Sal		DO	pH	Sal		DO	pH	Sal		DO	pH	Sal
Control		16.3	8.0	7.49	26	15.1	8.7	7.77	27	16.2	8.4	7.87	26	15.4	8.4	7.79	26	15.7	8.2	7.89	27
HSW-1	0.08	16.0	8.1	7.42	26	14.5	8.6	7.62	27	15.6	8.4	7.86	26	15.6	7.7	7.84	26	15.9	8.1	7.88	26
	0.15	16.0	8.0	7.43	27	14.5	6.6	7.51	27	15.5	7.4	7.80	27	15.6	6.5	7.80	27	15.7	8.1	7.85	27
	0.3	16.2	8.0	7.83	29	14.5	4.5	7.54	29	15.7	2.2	7.59	28	15.5	3.0	7.47	28	15.8	7.8	7.65	29
	0.6	16.2	8.0	7.51	26	14.5	4.1	7.51	27	15.9	2.3	7.56	26	15.6	2.7	7.49	26	15.7	7.4	7.93	27
	1.2	16.4	8.0	7.62	26	14.5	1.5	7.10	29	15.6	1.3	7.46	28	15.7	1.7	7.51	27	15.1	7.4	7.97	29
HSW-2	0.08	16.2	8.0	7.33	26	14.5	1.2	7.41	27	15.3	7.7	7.93	27	15.6	7.9	7.80	27	15.2	7.6	7.95	27
	0.15	16.4	8.0	7.34	27	14.5	1.6	7.42	27	15.5	7.7	7.96	27	15.7	7.3	7.77	27	15.0	7.8	7.95	27
	0.3	16.4	8.0	7.21	27	14.5	1.3	7.45	27	15.6	7.8	7.82	27	15.6	6.9	7.79	27	15.0	7.8	7.97	27
	0.6	16.0	8.0	7.21	26	15.7	1.3	7.42	27	16.2	3.0	7.52	27	15.7	2.7	7.47	27	16.2	6.6	7.71	27
	1.2	16.2	7.9	6.87	26	15.7	1.3	7.10	27	16.1	1.4	7.42	27	15.7	1.7	7.38	27	16.2	6.4	7.63	27
	Min	16.0	7.9	6.87	26	14.5	1.2	7.10	27	15.3	1.3	7.42	26	15.4	1.7	7.38	26	15.0	6.4	7.63	26
	Max	16.4	8.1	7.83	29	15.7	8.7	7.77	29	16.2	8.4	7.96	28	15.7	8.4	7.84	28	16.2	8.2	7.97	29

APPENDIX TABLE 11

Strongylocentrotus purpuratus
SUMMARY OF SURVIVAL AND DEVELOPMENT FOR THE ECHINODERM LARVAE
EFFLUENT TEST
Test Dates: 4/7-4/11/94

Concentration (%)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
Initial Counts	1	156		31.2			
	2	136		27.2			
	3	141		28.2			
	4	168		33.6			
	5	137		27.4			
	Mean			29.5			
Final Control	1	95	14	21.8		12.8	
	2	59	4	12.6		6.3	
	3	109	7	23.2		6.0	
	4	94	1	19.0		1.1	
	5	90	2	18.4		2.2	
	Mean			19.0	64.4	5.7	NA
HSW-1 0.08	1	45	32	15.4		41.6	
	2	63	53	23.2		45.7	
	3	66	43	21.8		39.4	
	4	76	38	22.8		33.3	
	5	78	40	23.6		33.9	
	Mean			21.4	72.4	38.8	0.0
0.15	1	0	79	15.8		100.0	
	2	0	48	9.6		100.0	
	3	0	44	8.8		100.0	
	4	0	89	17.8		100.0	
	5	0	99	19.8		100.0	
	Mean			14.4	48.7	100.0	24.4
0.3	1	0	50	10.0		100.0	
	2	0	53	10.6		100.0	
	3	0	57	11.4		100.0	
	4	0	84	16.8		100.0	
	5	0	58	11.6		100.0	
	Mean			12.1	40.9	100.0	36.4
0.6	1	0	66	13.2		100.0	
	2	0	85	17.0		100.0	
	3	0	74	14.8		100.0	
	4	0	112	22.4		100.0	
	5	0	57	11.4		100.0	
	Mean			15.8	53.4	100.0	17.1
1.2	1	0	106	21.2		100.0	
	2	0	115	23.0		100.0	
	3	0	92	18.4		100.0	
	4	0	60	12.0		100.0	
	5	0	114	22.8		100.0	
	Mean			19.5	66.0	100.0	100.0

APPENDIX TABLE 11 (Cont'd)

Strongylocentrotus purpuratus
SUMMARY OF SURVIVAL AND DEVELOPMENT FOR THE ECHINODERM LARVAE
EFFLUENT TEST
Test Dates: 4/7-4/11/94

Concentration (%)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
HSW-2							
	0.08						
	1	0	63	12.6		100.0	
	2	0	61	12.2		100.0	
	3	0	39	7.8		100.0	
	4	0	36	7.2		100.0	
	5	0	58	11.6		100.0	
	Mean			10.3	34.8	100.0	45.9
0.15							
	1	0	101	20.2		100.0	
	2	0	112	22.4		100.0	
	3	0	129	25.8		100.0	
	4	0	122	24.4		100.0	
	5	0	130	26.0		100.0	
	Mean			23.8	80.5	100.0	0.0
0.3							
	1	0	89	17.8		100.0	
	2	0	128	25.6		100.0	
	3	0	119	23.8		100.0	
	4	0	119	23.8		100.0	
	5	0	91	18.2		100.0	
	Mean			21.8	74.0	100.0	0.0
0.6							
	1	0	116	23.2		100.0	
	2	0	119	23.8		100.0	
	3	0	113	22.6		100.0	
	4	0	79	15.8		100.0	
	5	0	104	20.8		100.0	
	Mean			21.2	72.0	100.0	0.0
1.2							
	1	0	76	15.2		100.0	
	2	0	87	17.4		100.0	
	3	0	92	18.4		100.0	
	4	0	88	17.6		100.0	
	5	0	76	15.2		100.0	
	Mean			16.8	56.8	100.0	11.8

APPENDIX TABLE 12

Strongylocentrotus purpuratus

WATER QUALITY MEASUREMENTS FOR THE REFERENCE TOXICANT (COPPER) TEST

Test Dates: 4/7-4/11/94

Concentration (µg/L)	Day 0				Day 1				Day 2				Day 3				Day 4			
	°C	DO	pH	Sal	°C	DO	pH	Sal	°C	DO	pH	Sal	°C	DO	pH	Sal	°C	DO	pH	Sal
0.1	15.6	8.9	7.88	29	14.3	NT	NT	NT	14.2	8.1	7.97	29	14.4	8.4	8.01	29	15.0	7.6	7.98	29
0.32	15.8	8.9	7.90	29	14.3	NT	NT	NT	14.2	8.1	8.00	29	14.4	8.4	8.04	29	15.0	7.7	7.99	29
1.8	15.8	8.9	7.92	29	14.4	NT	NT	NT	14.3	8.3	8.02	29	14.5	8.3	8.06	29	14.9	7.9	8.00	29
18	15.8	9.1	7.80	28	14.3	NT	NT	NT	14.2	8.3	8.01	28	14.5	8.3	8.06	29	15.0	7.9	8.00	29
56	15.8	9.1	7.86	26	14.4	NT	NT	NT	14.2	8.6	8.02	25	14.5	8.3	8.06	29	15.0	8.0	8.01	25
Min	15.6	8.9	7.80	26	14.3				14.2	8.1	7.97	25	14.4	8.3	8.01	29	14.9	7.6	7.98	25
Max	15.8	9.1	7.92	29	14.4				14.3	8.6	8.02	29	14.5	8.4	8.06	29	15.0	8.0	8.01	29

Note: NT = Not taken.

APPENDIX TABLE 13

Strongylocentrotus purpuratus
SUMMARY OF SURVIVAL AND DEVELOPMENT FOR THE ECHINODERM LARVAE
REFERENCE TOXICANT (Copper) TEST
Test Dates: 4/7-4/11/94

Concentration (µg/L)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
Copper 0.1	1	78	14	18.4		15.2	
	2	86	19	21.0		18.1	
	3	86	12	19.6		12.2	
	Mean			19.7	66.7	15.2	0.0
0.32	1	26	1	5.4		3.7	
	2	33	1	6.8		2.9	
	3	96	0	19.2		0.0	
	Mean			10.5	35.5	2.2	44.9
1.8	1	69	4	14.6		5.5	
	2	60	2	12.4		3.2	
	3	96	4	20.0		4.0	
	Mean			15.7	53.1	4.2	17.5
18	1	3	51	10.8		94.4	
	2	0	31	6.2		100.0	
	3	0	28	5.6		100.0	
	Mean			7.5	25.5	98.1	60.4
56	1	0	38	7.6		100.0	
	2	0	24	4.8		100.0	
	3	0	48	9.6		100.0	
	Mean			7.3	24.9	100.0	61.4

APPENDIX TABLE 14

Mytilus edulis
WATER QUALITY MEASUREMENTS FOR THE EFFLUENT TEST
Test Dates: 4/7-4/9/94

Concentration		Day 0				Day 1		Day 2		
(%)	Rep	°C	DO	pH	Sal	°C	°C	DO	pH	Sal
Control	1	16.3	8.0	7.49	26	14.8	16.0	7.2	7.79	26
	2					14.6	16.0	7.2	7.82	26
	3					14.5	16.0	7.5	7.82	26
	4					14.7	16.0	7.5	7.88	26
	5					14.8	16.0	7.6	7.96	26
HSW-1										
0.08	1	16.0	8.1	7.42	26	14.5	16.0	7.6	7.68	26
	2					14.5	16.0	7.5	7.65	26
	3					14.4	16.1	7.3	7.67	26
	4					14.5	16.0	7.2	7.66	26
	5					14.5	16.1	7.1	7.66	26
0.15	1	16.0	8.0	7.43	27	14.5	16.0	4.0	7.46	26
	2					14.4	16.0	4.0	7.40	26
	3					14.4	16.0	3.8	7.38	26
	4					14.4	16.0	3.8	7.38	26
	5					14.5	16.0	3.6	7.40	26
0.3	1	16.2	8.0	7.83	29	14.4	16.0	2.0	7.44	28
	2					14.5	16.0	2.0	7.52	28
	3					14.5	16.0	1.8	7.54	28
	4					14.4	16.0	1.8	7.56	28
	5					14.5	16.0	1.5	7.55	28
0.6	1	16.2	8.0	7.51	26	14.5	16.0	1.6	7.56	26
	2					14.5	16.0	1.7	7.58	26
	3					14.5	16.0	1.7	7.60	26
	4					14.6	16.1	2.1	7.61	26
	5					14.5	16.1	2.0	7.60	26
1.2	1	16.4	8.0	7.62	26	14.4	16.0	4.2	7.62	26
	2					14.5	16.0	4.4	7.67	26
	3					14.5	16.0	4.3	7.64	26
	4					14.5	16.1	4.5	7.67	26
	5					14.5	16.1	4.6	7.83	26
Min		16.0	8.0	7.42	26	14.4	16.0	1.5	7.38	26
Max		16.4	8.1	7.83	29	14.8	16.1	7.6	7.96	28

APPENDIX TABLE 14 (Cont'd)

Mytilus edulis
WATER QUALITY MEASUREMENTS FOR THE EFFLUENT TEST
Test Dates: 4/7-4/9/94

Concentration		Day 0				Day 1		Day 2		
(%)	Rep	°C	DO	pH	Sal	°C	°C	DO	pH	Sal
HSW-2										
0.08	1	16.2	8.0	7.33	26	14.5	16.0	7.4	7.93	26
	2					14.6	16.0	7.7	7.92	26
	3					14.5	16.0	7.5	7.95	26
	4					14.5	16.1	7.5	7.97	26
	5					14.5	16.1	7.6	7.98	27
0.15	1	16.4	8.0	7.34	27	14.5	16.0	7.8	7.91	26
	2					14.5	16.0	8.0	7.94	26
	3					14.4	16.1	8.0	7.94	26
	4					14.5	16.1	7.9	7.86	26
	5					14.5	16.1	7.7	7.85	26
0.3	1	16.4	8.0	7.21	27	14.5	16.0	7.7	7.83	26
	2					14.5	16.0	7.7	7.86	26
	3					14.5	16.0	7.7	7.77	26
	4					14.5	16.1	7.6	7.59	26
	5					14.5	16.1	7.2	7.62	26
0.6	1	16.0	8.0	7.21	26	14.5	16.0	1.7	7.56	26
	2					14.6	16.1	1.7	7.53	26
	3					14.5	16.1	1.8	7.51	26
	4					14.6	16.1	1.8	7.51	26
	5					14.5	16.1	1.8	7.50	26
1.2	1	16.2	7.9	6.87	26	14.5	16.0	2.0	7.47	26
	2					14.5	16.1	1.7	7.37	26
	3					14.5	16.1	1.6	7.39	26
	4					14.5	16.1	2.0	7.42	26
	5					14.5	16.1	2.0	7.45	26
	Min	16.0	7.9	6.87	26	14.4	16.0	1.6	7.37	26
	Max	16.4	8.0	7.34	27	14.6	16.1	8.0	7.98	27

APPENDIX TABLE 15

Mytilus edulis
SUMMARY OF RESULTS FOR BIVALVE LARVAE BIOASSAY
Test Dates: 4/7-4/9/94

Concentration (%)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
Initial Counts	1	129		25.8			
	2	95		19.0			
	3	102		20.4			
	4	76		15.2			
	5	115		23.0			
	Mean			20.7			
Final Control	1	103	13	23.2		11.2	
	2	97	3	20.0		3.0	
	3	86	5	18.2		5.5	
	4	83	5	17.6		5.7	
	5	106	7	22.6		6.2	
	Mean			20.3	98.2	6.3	NA
HSW-1 0.08	1	22	61	16.6		73.5	
	2	2	78	16.0		97.5	
	3	0	72	14.4		100.0	
	4	0	77	15.4		100.0	
	5	5	67	14.4		93.1	
	Mean			15.4	74.2	92.8	24.3
0.15	1	0	74	14.8		100.0	
	2	0	76	15.2		100.0	
	3	0	64	12.8		100.0	
	4	0	86	17.2		100.0	
	5	0	61	12.2		100.0	
	Mean			14.4	69.8	100.0	28.9
0.3	1	0	139	27.8		100.0	
	2	0	120	24.0		100.0	
	3	0	133	26.6		100.0	
	4	0	91	18.2		100.0	
	5	0	82	16.4		100.0	
	Mean			22.6	100.0	100.0	0.0
0.6	1	0	73	14.6		100.0	
	2	0	133	26.6		100.0	
	3	0	90	18.0		100.0	
	4	0	96	19.2		100.0	
	5	0	93	18.6		100.0	
	Mean			19.4	93.7	100.0	4.4
1.2	1	0	90	18.0		100.0	
	2	0	75	15.0		100.0	
	3	0	87	17.4		100.0	
	4	0	80	16.0		100.0	
	5	0	91	18.2		100.0	
	Mean			16.9	81.7		16.7

APPENDIX TABLE 15 (Cont'd)

Mytilus edulis
SUMMARY OF RESULTS FOR BIVALVE LARVAE BIOASSAY
Test Dates: 4/7-4/9/94

Concentration (%)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
HSW-2 0.08	1	0	109	21.8		100.0	
	2	1	84	17.0		98.8	
	3	0	100	20.0		100.0	
	4	0	110	22.0		100.0	
	5	0	95	19.0		100.0	
	Mean			20.0	96.4	99.8	1.7
0.15	1	0	100	20.0		100.0	
	2	0	90	18.0		100.0	
	3	0	111	22.2		100.0	
	4	0	89	17.8		100.0	
	5	0	115	23.0		100.0	
	Mean			20.2	97.6	100.0	0.5
0.3	1	0	82	16.4		100.0	
	2	0	101	20.2		100.0	
	3	0	97	19.4		100.0	
	4	0	89	17.8		100.0	
	5	0	104	20.8		100.0	
	Mean			18.9	91.4	100.0	6.8
0.6	1	0	144	28.8		100.0	
	2	0	128	25.6		100.0	
	3	0	94	18.8		100.0	
	4	0	103	20.6		100.0	
	5	0	119	23.8		100.0	
	Mean			23.5	100.0	100.0	0.0
1.2	1	0	81	16.2		100.0	
	2	0	94	18.8		100.0	
	3	0	104	20.8		100.0	
	4	0	88	17.6		100.0	
	5	0	87	17.4		100.0	
	Mean			18.2	87.7	100.0	10.5

APPENDIX TABLE 16

Mytilus edulis
WATER QUALITY MEASUREMENTS
FOR THE REFERENCE TOXICANT (COPPER) TEST
Test Dates: 4/7-4/9/94

Concentration		Day 0				Day 1		Day 2		
$\mu\text{g/L}$	Rep	$^{\circ}\text{C}$	DO	pH	Sal	$^{\circ}\text{C}$	$^{\circ}\text{C}$	DO	pH	Sal
0.56	1	15.8	9.2	7.91	30	14.3	14.0	7.7	7.95	28
	2					14.3	14.0	7.8	7.96	29
	3					14.3	14.0	7.9	7.96	29
3.2	1	15.7	8.9	7.91	29	14.3	14.1	7.9	7.96	28
	2					14.3	14.0	7.9	7.96	29
	3					14.2	14.0	8.1	7.96	29
10	1	15.6	8.7	7.92	29	14.3	14.0	8.0	7.96	28
	2					14.4	14.1	8.0	7.97	28
	3					14.3	14.1	8.1	7.97	28
32	1	15.6	9.7	7.78	26	14.3	14.0	8.0	7.97	26
	2					14.3	14.1	8.1	7.96	26
	3					14.3	14.1	8.1	7.95	26
56	1	15.8	9.1	7.86	26	14.4	14.0	8.3	7.95	25
	2					14.3	14.0	8.1	7.96	25
	3					14.4	14.0	8.1	7.96	25
Min		15.6	8.7	7.78	26	14.2	14.0	7.7	7.95	25
Max		15.8	9.7	7.92	30	14.4	14.1	8.3	7.97	29

APPENDIX TABLE 17

Mytilus edulis

SUMMARY OF RESULTS FOR THE BIVALVE LARVAE

REFERENCE TOXICANT (COPPER) BIOASSAY

Test Dates: 4/7-4/9/94

Concentration ($\mu\text{g/L}$)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
0.56	1	92	5	19.4		5.2	
	2	76	3	15.8		3.8	
	3	86	6	18.4		6.5	
	Mean			17.9	86.3	5.2	12.0
3.2	1	99	24	24.6		19.5	
	2	95	22	23.4		18.8	
	3	89	17	21.2		16.0	
	Mean			23.1	100.0	18.1	0.0
10	1	88	16	20.8		15.4	
	2	11	91	20.4		89.2	
	3	29	45	14.8		60.8	
	Mean			18.7	90.2	55.1	8.0
32	1	0	34	6.8		100.0	
	2	0	12	2.4		100.0	
	3	0	50	10.0		100.0	
	Mean			6.4	30.9	100.0	68.5
56	1	0	0	0.0		100.0	
	2	0	6	1.2		100.0	
	3	0	13	2.6		100.0	
	Mean			1.3	6.1	100.0	93.8

Copy to Vance Long/Eugenia
for review



9 August 1996

107091.EL.96

Patricia N.N. Young
American Samoa Program Manager
Office of Pacific Islands
and Native American Programs
U.S. Environmental Protection Agency
75 Hawthorne Street (E-4)
San Francisco, California 94105

Sheila Wiegman
American Samoa
Environmental Protection Agency
American Samoa Government
Pago Pago, American Samoa 96799

Dear Pat and Shiela:

**Subject: Joint Cannery Outfall Effluent Bioassay Testing
Results of Bioassay Tests 6 (Feb 96) and 7 (Mar 96)**

Enclosed are two copies each of technical memoranda describing the results of the sixth and seventh episodes of whole effluent bioassay testing done under StarKist Samoa and VCS Samoa Packing NPDES permit requirements. As you know the test scheduled for October 1995 was delayed until early February 1996 because of shipping problems as described in the memorandum. For the tests done on the February 1996 samples, we performed bioassays on both *Penaeus vannamei* and *Mysidopsis bahia* for reasons described in the report. For the March 1996 sample, we used only a single species, *Penaeus vannamei*. This is the species we will use in the future, unless availability requires us to substitute *Mysidopsis bahia*. Unless USEPA or ASEPA have specific concerns, we will continue performing the tests as described in these reports. I have not sent copies directly to anyone else at USEPA since I am not sure who you will want to further review the reports (I have included an extra copy for USEPA to forward as appropriate). The next test is scheduled for September/October 1996.

If you have any questions please feel free to call me at your convenience.

Sincerely,

CH2M HILL

Steven L. Costa
Project Manager

cc: USEPA Region IX, (1 extra copy of enclosure for distribution)
Norman Wei, StarKist Seafood Company (1 copy of enclosure)
James Cox, Van Camp Seafood Company (1 copy of enclosures)
Barry Mills, StarKist Samoa, Inc. (1 copy of enclosures)
Bill Perez, VCS Samoa Packing Company (1 copy of enclosures)
Kurt Kline, Advanced Biological Testing (1 copy of enclosure)
Karen Glatzel, Glatzel and Associates

AUG 13 1996
RECEIVED
Shiela

TECHNICAL MEMORANDUM

PREPARED FOR: StarKist Samoa, Inc.
VCS Samoa Packing Company, Inc.

PREPARED BY: Steve Costa/CH2M HILL/SFO
Karen A. Glatzel/Glatzel & Associates

DATE: 9 August 1996

SUBJECT: Bioassay Testing of Effluent
March 1996 Sampling

PROJECT: 107091.EL.96

Purpose

This memorandum presents the results of the effluent bioassay testing of the Joint Cannery Outfall effluent sample that was collected in March 1996. This is the seventh of the required semi-annual tests. Separate technical memoranda are being prepared to describe the results of concurrent effluent chemistry testing.

Study Objectives

Section D.1 of the StarKist Samoa and VCS Samoa Packing NPDES permits requires that semi-annual definitive acute bioassays (96-hour static bioassays) be conducted on the cannery effluent. The purpose of these bioassays is to determine whether, and at what effluent concentration, acute toxicity may be detected for the effluent.

U.S. EPA has conducted a number of reviews of the effluent sampling, analysis, and bioassay tests. All comments from U.S. EPA have been incorporated into either the Standard Operating Procedures or have been incorporated into the procedures by the laboratory doing the test, Advanced Biological Testing, Inc., as documented in previous reports.

The bioassays were originally specified to be conducted using the white shrimp, *Penaeus vannamei* (postlarvae). In the event *Penaeus vannamei* are not available at the time of the tests, a substitute species, *Mysidopsis bahia*, has been approved by U.S. EPA (CH2M HILL, 26 January 1995). A substitution was not necessary for the March 1996 sampling, and *Penaeus vannamei* were used.

The acute bioassay effluent sampling must be concurrent with effluent sampling for priority pollutant chemical analysis. Effluent samples are to be collected as 24-hour composite

samples. The effluent acute bioassay was conducted using a combined composite effluent sample made up from the composite effluent samples from the StarKist Samoa and VCS Samoa Packing facilities, as approved by EPA. This combined effluent bioassay is representative of the wastewater discharged from the joint cannery outfall to Pago Pago Harbor.

Effluent Sampling Methods

Between 1200 on March 13 and 0900 on March 14, 1996, 24-hour, flow-weighted, composite samples of final effluent were collected from both the StarKist Samoa and VCS Samoa Packing treatment plant discharges. Samples were collected from the established effluent sampling sites following the routine composite sample collection schedule for the plants. Detailed sampling procedures were provided in the March 1995 technical memorandum as Attachment II.

A total of eight grab samples were collected into pre-cleaned 1-gallon plastic cubitainers at each plant. Samples were collected at approximately three-hour intervals over a 24 hour period. The samples were stored on ice until the completion of the 24-hour sampling period. After all samples were collected a flow-proportioned composite sample was prepared. The grab sample collection times and the relative effluent volumes calculated from plant flow records are summarized in Table 1. The relative effluent volumes were used to prepare the final composite sample, which was used to fill the sample container shipped to the laboratory for testing.

A 5-gallon cubitainer containing the composite sample was packed on ice in an ice chest for shipment to the laboratory. Sample chain of custody forms were completed and then sealed into zip-lock bags and taped inside the lid of the ice chest. Samples were shipped via DHL on flights from Pago Pago to Honolulu and then to San Francisco. Samples were delivered to the testing laboratory on 18 March 1996.

Bioassay Testing Procedures

The bioassay tests were conducted by Advanced Biological Testing Inc., Tiburon, California. The testing procedures and results of the bioassay tests are provided in "*Results of a Bioassay Conducted on an Effluent Sample from the Joint Cannery Outfall in American Samoa using Penaeus vannamei*" dated 1 April 1996 and included as Attachment I. This report summarizes the 96-hour acute bioassay test conducted with reference to U.S. EPA (1991) document EPA/600/4-90/027 as the source of methods for conducting the test.

Effluent Bioassay Testing
March 1996 Sampling
StarKist Samoa/VCS Samoa Packing

The bioassay tests were conducted considering and including U.S. EPA's comments on previous bioassay tests, as documented in previous reports. A brine control was run and a comparison was made with the dilution water "laboratory control". The test organisms were required to be 1 to 5 days old, with a 24-hour range in age and that test temperature be $20 \pm 1^\circ\text{C}$ or $25 \pm 1^\circ\text{C}$. The penaeids were postlarvae (8 to 10 mm) tested at $20 \pm 1^\circ\text{C}$.

Because of the demonstrated potential for a lethal immediate dissolved oxygen demand (IDOD), discussed and documented in previous technical memoranda describing the first two bioassay tests, each bioassay test chamber was continuously aerated during the bioassay tests to maintain adequate levels of dissolved oxygen (DO). Bioassay tests were carried out for effluent concentrations of 50, 25, 12.5, 6.25, and 3.1 % as vol:vol dilutions in seawater.

Water quality was monitored daily with parameters measured including DO, pH, salinity, temperature, and ammonia. Additionally, a reference toxicant of sodium dodecyl sulfonate (SDS) was made up of a 2-gram per liter stock solution in distilled water and run at concentrations of 100, 50, 25, 12.5, and 6.25 mg/L in 30 ppt seawater for a 96-hour test.

Results

The results of the bioassay tests are summarized as follows:

***Penaeus vannamei* Effluent Bioassay.** All results from the bioassay tests are included in Attachment I. The results of the penaeid bioassay tests indicate the LC_{50} for the effluent tested was 44.4 percent. The No Observable Effects Concentration (NOEC) for the 96-hour bioassay was 25 percent and the Least Observable Effects Concentration (LOEC) was 50 percent. The calculated value of toxicity units (TU) was 4.

***Penaeus vannamei* Reference Toxicant Bioassay.** The reference toxicant had a LC_{50} of 42.9 mg/l. The laboratory mean was 26.39 mg/l with the data falling within two standard deviations of the laboratory mean, indicating normal to slightly lower than normal sensitivity.

Discussion

Table 2 summarizes the results of the effluent bioassay tests for the samples collected in the March 1996 sampling compared to the previous bioassay tests. The NOEC and LC_{50} are within the range obtained for previous penaeid tests. The penaeid survival in this test compares well to the survival of penaeids in February 1996 test.

Conclusions

The bioassay tests for the Joint Cannery Outfall effluent for March 1996 are not considered to be of concern. As discussed in the previous bioassay test reports on the effluent, the time scale of the mixing of the effluent with the receiving water is on the order of minutes to seconds to achieve dilutions that will eliminate possible toxic effects as reflected by the bioassay results. For example, an NOEC of 25% in this test corresponds to a dilution of 4:1 which is achieved in a few seconds. The discharge is located in about 180 feet of water and the effluent toxicity tests indicate that the discharge is diluted to non-toxic levels immediately after discharge and well within the initial dilution plume.

Effluent Bioassay Testing
 March 1996 Sampling
 StarKist Samoa/VCS Samoa Packing

Table 1 StarKist Samoa and VCS Samoa Packing 24-hour Composite Effluent Sample for Bioassay Testing 13-14 March 1996						
Grab Sample Number	VCS Samoa Packing		StarKist Samoa		VCS Sa- moa Packing Percent of Total Flow	StarKist Samoa Percent of Total Flow
	Sampling Date and Time	Effluent Flow Rate (mgd)	Sampling Date and Time	Effluent Flow Rate (mgd)		
1	3/13/96 1200	0.56	2/1/96 1200	1.19	3.7	8.0
2	1500	0.52	1500	1.26	3.5	8.4
3	1800	0.52	1800	1.26	3.5	8.4
4	2100	0.50	2100	1.33	3.3	8.9
5	2400	0.52	2400	1.36	3.5	9.1
6	3/14/96 0300	0.60	2/2/96 0300	1.40	4.0	9.4
7	0600	0.60	0600	1.05	4.0	7.0
8	0900	0.52	0900	1.75	3.5	11.7
Total		4.34		10.6	29.0	70.9
Mean		0.54		1.33		

Effluent Bioassay Testing
 March 1996 Sampling
 StarKist Samoa/VCS Samoa Packing

Table 2 StarKist Samoa and VCS Samoa Packing Combined Effluent Bioassay Results				
Date	Species	Parameters		
		LC 50	NOEC	LOEC
2/93	<i>Penaeus vannami</i>	4.8% ¹	3.1%	6.25%
10/93	<i>Penaeus vannami</i>	15.67%	3.1%	6.25%
2/94	<i>Penaeus vannami</i>	15.76%	< 1.6%	1.6%
10/94	<i>Mysidopsis bahia</i> ²	31.2%	25%	50%
3/95	<i>Penaeus vannami</i>	14.8%	6.25%	12.5%
3/95	<i>Mysidopsis bahia</i> ³	10.8%	6.25%	12.5%
2/96	<i>Penaeus vannami</i>	> 50%	> 50%	> 50%
2/96	<i>Mysidopsis bahia</i> ³	28.36%	12.5%	25%
3/96	<i>Penaeus vannami</i>	44.4%	25%	50%
¹ The February 1993 samples were not aerated until after the first day of the test. For subsequent tests the samples were aerated for the entire duration of the tests. ² <i>Mysidopsis bahia</i> substitutes as <i>Penaeus vannami</i> not available, as directed by U. S. EPA. ³ <i>Mysidopsis bahia</i> used in addition to <i>Penaeus vannami</i> as described in text. Only one species is required by the permit conditions.				

ATTACHMENT I

**LABORATORY REPORT
Advanced Biological Testing
96-hour Acute Bioassay**

**JOINT CANNERY OUTFALL EFFLUENT SAMPLE
March 13-14, 1996**

**RESULTS OF BIOASSAYS CONDUCTED ON
AN EFFLUENT SAMPLE
FROM THE JOINT CANNERY OUTFALL
IN AMERICAN SAMOA
Using *Penaeus vannamei***

Prepared for:

CH2M Hill California, Inc.
1111 Broadway
Oakland, CA 94607
Project # PDX 30702

Prepared by:

Advanced Biological Testing Inc.
98 Main St., # 419
Tiburon, Ca. 94920

April 1, 1996

Ref: 9611

INTRODUCTION

At the request of CH2M Hill (Project # PDX 30702), Advanced Biological Testing conducted a four day effluent bioassay test on *Penaeus vannami* using effluents collected from the joint cannery outfall at the Starkist and Van Camp tuna canneries in American Samoa. The study was run using methods generally specified in EPA 1991. *Penaeus vannami* is the test species designated in the NPDES permit.

The study was conducted at the Advanced Biological Testing Laboratory in Tiburon, California, and was managed by Mr. Mark Fisler.

2.1 EFFLUENT SAMPLING

The effluents were sampled on March 15, 1996 by cannery personnel under the supervision of CH2M Hill. The sample was received by the laboratory on March 18, 1996. One five gallon carboy was provided, maintained in an ice-filled cooler from the date of sampling until laboratory receipt. The sample was at 5°C upon receipt.

2.2 SAMPLE PREPARATION

The salinity of the effluent sample was 14 ppt. The effluents required salinity adjustment to 30 ppt. The effluent salinity was increased to 30 ppt with 100 ppt natural seawater brine. The brine was made from frozen Bodega Bay seawater. Due to the dilution of the effluent with the brine solution, the initial maximum concentration of effluent was 81%. The highest initial test concentration was made by diluting the 81% effluent with Bodega Bay seawater to an actual effluent concentration of 50%.

The effluents were tested at an actual effluent concentration series of 50%, 25%, 12.5%, 6.25%, and 3.1% as a vol:vol dilutions in seawater. A brine control was run to assess the potential toxicity from the added brine. The diluent and the control water were filtered seawater from Bodega Bay. The dilutions were brought to the test temperature ($20 \pm 2^{\circ}\text{C}$) and aerated continuously. These effluents have been shown to have an increasing biological oxygen demand, with a significant peak at 10-14 hours after test initiation. Previous testing of this effluent without initial aeration has demonstrated significant toxicity at 24 hours (or before); therefore aeration was carried out from the beginning of the test. According to EPA methods the effluents were renewed with effluents held under refrigeration from test initiation on Day 2.

A reference toxicant was run using concentrations provided by the EPA. The toxicant was sodium dodecyl sulfonate (SDS) made up as a 2 grams per liter stock solution in distilled water. The tested concentrations were set at 100, 50, 25, 12.5 and 6.25 mg/L in 30 ppt seawater.

2.3 TESTING PROCEDURES

The bioassay was carried out on post-larval *Penaeus vannamei* provided by Brezina and Associates. The animals were air-shipped from Hawaii and were received at ABT on March 19, 1996. Five replicates of each concentration were tested with ten animals per replicate. Water quality was monitored daily as initial quality on Day 0 and final water quality on Days 1-4. Parameters measured included dissolved oxygen, pH, salinity, total ammonia, and temperature.

2.4 STATISTICAL ANALYSIS

At the conclusion of the test, the survival data were evaluated statistically using ToxCalc™ to determine ECp, NOEC, and TU values where appropriate. ToxCalc™ is a comprehensive statistical application that follows standard guidelines for acute and chronic toxicity data analysis. Statistical effects can be measured by the ECp, the estimated concentration that causes any effect, either lethal (LC) or sublethal (IC), on p% of the test population. The LCp is the point estimate of the concentration at which a lethal effect is observed in p% of the test organisms. ECp values include 95% confidence limits if available.

The NOEC (No Observable Effect Concentration) is the highest tested concentration at which mortality and other sublethal measured effects are not significantly different from the same parameters in the control. TU (Toxicity Units) are calculated as 100%/NOEC.

3.0
RESULTS

3.1 INTRODUCTION

Table 1 summarizes the test parameters and conditions. The results of the effluent and reference toxicant bioassays and the water quality monitoring for both sets of tests are presented in Tables 2 through 6.

3.2 TEST RESULTS

Water quality measurements were within the acceptable limits provided in EPA 1991. Temperature was maintained at $20 \pm 1^{\circ}\text{C}$; pH remained relatively stable, and the salinity increased slightly as would be expected in a static test (Tables 2 and 3). Aeration was maintained in all chambers for the duration of the test. The test solutions were renewed with reserved effluent at 48 hrs (Day 2).

Ammonia was 3.09 ppm in the 50% effluent at test initiation, and increased to 3.21 ppm by Day 4. The LC50 for the effluent was 44.4%. There was significant mortality at the 50% concentrations compared to the control (Table 3). The NOEC was 25%, and the LOEC was 50%. The TU was 4.

The reference toxicant test had an LC50 of 42.9 mg/L (Tables 5 and 6). The laboratory mean was 26.39 mg/L and the data, while higher than the mean, is within two standard deviation of the laboratory mean, indicating normal, though lower sensitivity.

TABLE 1

**Bioassay Procedure And Organism Data
For the Survival Bioassay
Using *Penaeus vannamei* (U.S. EPA 1991)**

<u>Parameter</u>	<u>Data</u>
<u>Sample Identification</u>	
Sample ID(s)	960318-1
Date Sampled	3/15/96
Date Received at ABT	3/18/96
Volume Received	Five gallons
Sample Storage Conditions	4°C in the dark
<u>Test Species</u>	
Supplier	J. Brezina and Associates, Dillon Beach, Ca
Collection location	Hawaii
Date Acquired	3/19/96
Acclimation Time	Used immediately
Acclimation Water	Shipping water
Acclimation Temperature	20±2°C
Age group	Post larvae (approximately 8-10 mm)
<u>Test Procedures</u>	
Type; Duration	Acute, static/renewal at 48 hours
Test Dates	3/19 - 23/96
Control Water	Bodega Bay seawater
Test Temperature	20 ± 2°C
Test Photoperiod	16L : 8 D
Salinity	30± 2 ppt
Test Chamber	1000 mL jars
Animals/Replicate	10
Exposure Volume	500 mL
Replicates/Treatment	5
Feeding	Brine shrimp (24 hr old nauplii)
Deviations from procedures	Aerated continuously

Advanced Biological Testing Inc.

TABLE 2

Penaeus vannamei
INITIAL WATER QUALITY MEASUREMENTS
FOR EFFLUENT TEST
Test Dates: 3/19/96 - 3/23/96

Concentration (%)	Day 0					Day 2				
	pH	DO	NH 3	°C	Sal	pH	DO	NH 3	°C	Sal
Control	8.00	7.4	0.01	20.6	29	8.02	8.0	0.02	18.3	30
Brine	8.03	7.4	0.01	20.3	29	8.01	8.0	0.02	18.5	29
3.1	7.91	7.4	0.18	20.4	29	7.84	8.0	0.22	18.6	29
6.25	7.80	7.4	0.37	20.5	29	7.67	7.6	0.42	19.0	29
12.5	7.67	7.4	0.74	20.5	29	7.35	7.4	0.82	18.9	29
25	7.47	7.4	1.51	20.6	29	7.56	8.0	1.62	18.9	29
50	7.54	7.3	3.09	20.6	29	7.33	7.4	3.21	18.6	29
Min	7.47	7.3	0.01	20.3	29	7.33	7.4	0.02	18.3	29
Max	8.03	7.4	3.09	20.6	29	8.02	8.0	3.21	19.0	30

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TABLE 3

Penaeus vannamei

FINAL WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST

Concentration (%)	Rep	Day 1				Day 2				Day 3				Day 4			
		pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal
Control	1	8.06	7.0	19.1	30	8.05	7.5	19.1	30	8.14	7.6	18.5	30	8.08	7.8	18.0	31
	2	8.06	7.0	18.9	30	8.05	7.4	18.9	30	8.13	7.6	18.3	30	8.09	7.7	18.0	31
	3	8.07	7.1	18.9	30	8.06	7.4	18.9	30	8.13	7.7	18.3	30	8.09	7.8	18.0	31
	4	8.05	7.1	18.9	30	8.03	7.4	18.9	30	8.10	7.7	18.2	30	8.06	7.8	18.0	31
	5	8.08	7.2	18.6	30	8.09	7.6	18.7	30	8.15	7.9	18.0	30	8.12	7.8	18.0	31
Brine Control	1	8.19	7.2	19.0	30	8.19	7.7	19.0	30	8.29	7.9	18.4	30	8.25	7.8	18.1	31
	2	8.17	7.2	18.9	30	8.18	7.6	18.9	30	8.29	7.8	18.2	30	8.23	7.8	18.0	30
	3	8.18	7.2	18.9	30	8.17	7.6	18.9	30	8.29	7.8	18.2	30	8.24	7.8	18.0	30
	4	8.18	7.2	18.9	30	8.17	7.6	18.9	30	8.29	7.8	18.2	30	8.24	7.8	18.0	30
	5	8.19	7.3	18.9	30	8.19	7.6	18.9	30	8.30	8.0	18.2	30	8.25	7.8	18.0	30
3.1	1	8.02	7.2	19.1	30	8.07	7.7	19.1	30	8.15	7.9	18.4	30	8.14	8.0	18.0	30
	2	8.05	7.2	18.9	30	8.11	7.6	18.9	30	8.18	8.0	18.2	30	8.15	7.9	18.1	30
	3	8.04	7.2	18.9	30	8.10	7.6	18.9	30	8.16	7.8	18.2	30	8.14	7.9	18.0	30
	4	8.02	7.2	18.9	30	8.08	7.6	18.9	30	8.15	7.8	18.3	30	8.13	7.9	18.0	30
	5	8.06	7.2	18.9	30	8.12	7.6	18.9	30	8.18	7.8	18.3	30	8.16	7.9	18.0	30
6.25	1	8.02	7.2	19.0	30	8.10	7.7	19.1	30	8.16	7.9	18.5	30	8.16	8.0	18.0	30
	2	8.01	7.2	18.9	30	8.11	7.6	19.0	30	8.17	7.9	18.4	30	8.16	7.9	18.0	30
	3	7.99	7.1	18.9	30	8.11	7.6	18.9	30	8.17	7.8	18.3	30	8.18	7.9	18.1	30
	4	7.96	7.0	18.9	30	8.08	7.6	19.0	30	8.12	7.8	18.4	30	8.15	7.8	18.0	30
	5	7.89	6.8	18.9	30	8.04	7.6	18.9	30	8.06	7.8	18.4	30	8.11	7.8	18.1	30
12.5	1	7.96	7.0	19.1	30	8.09	7.6	19.1	30	8.14	7.8	18.6	30	8.19	8.0	18.0	30
	2	7.98	7.0	19.0	30	8.12	7.6	19.1	30	7.82	6.9	18.6	30	7.97	7.6	18.1	30
	3	7.90	6.9	19.0	30	8.03	7.6	19.1	30	7.96	7.4	18.5	30	8.08	7.6	18.0	30
	4	7.98	7.0	19.0	30	8.10	7.6	19.1	30	8.11	7.6	18.5	30	8.19	7.8	18.0	30
	5	8.03	7.0	19.0	30	8.14	7.6	18.9	30	8.18	7.7	18.3	30	8.22	7.8	18.0	30
25	1	8.02	7.0	19.2	30	8.12	7.6	19.2	30	8.20	7.8	18.6	30	8.25	7.9	18.2	31
	2	8.06	6.9	19.1	30	8.14	7.4	19.1	30	8.20	7.6	18.5	30	8.26	7.8	18.1	31
	3	8.11	7.0	18.9	30	8.19	7.5	19.1	30	8.24	7.6	18.4	30	8.28	7.8	18.0	31
	4	8.06	7.0	18.9	30	8.15	7.4	19.1	30	8.21	7.7	18.4	30	8.26	7.8	18.0	31
	5	8.11	7.0	18.9	30	8.18	7.4	19.0	30	8.25	7.4	18.4	30	8.28	7.8	18.0	31
50	1	8.01	6.8	18.9	30	8.16	7.2	18.9	30	8.19	7.4	18.4	30	8.31	7.8	18.0	31
	2	8.07	6.8	18.9	30	8.24	7.4	18.9	30	8.27	7.4	18.3	30	8.37	7.6	18.1	31
	3	8.10	6.8	18.9	30	8.23	7.4	18.9	30	8.25	7.6	18.3	30	8.37	7.6	18.0	31
	4	8.13	7.0	18.7	30	8.29	7.6	18.8	30	8.28	7.6	18.2	30	8.39	7.7	18.1	31
	5	8.11	6.8	18.9	30	8.27	7.6	18.9	30	8.26	7.6	18.4	30	8.39	7.8	18.0	31
Min		7.89	6.8	18.6	30	8.03	7.2	18.7	30	7.82	6.9	18.0	30	7.97	7.6	18.0	30
Max		8.19	7.3	19.2	30	8.29	7.7	19.2	30	8.30	8.0	18.6	30	8.39	8.0	18.2	31

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TABLE 4

Penaeus vannamei
SURVIVAL DATA FOR EFFLUENT TEST

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
Brine Control	1	10	8	8	8	8	80	94.0
	2	10	10	10	10	9	90	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
3.1	1	12	12	12	12	12	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
6.25	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
12.5	1	10	10	10	10	10	100	88.0
	2	10	10	10	7	4	40	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
25	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
50	1	10	10	10	10	1	10	36.0
	2	10	10	10	10	2	20	
	3	10	10	10	10	7	70	
	4	10	10	10	10	4	40	
	5	10	10	10	10	4	40	

TABLE 5

Penaeus vannamei
WATER QUALITY MEASUREMENTS
FOR REFERENCE TOXICANT (S.D.S) TEST

Concentration (mg/L)	Rep	Day 0				Day 1				Day 2				Day 3				Day 4			
		pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal
Control	1	8.00	7.4	20.4	29	7.88	6.0	19.2	30	7.70	5.6	19.3	30	7.63	5.4	18.7	30	7.66	6.2	18.3	30
	2					7.91	6.0	19.1	30	7.79	6.0	19.1	30	7.80	6.2	18.5	30	7.77	6.6	18.0	30
	3					7.91	6.0	19.1	30	7.80	6.2	19.1	30	7.81	6.3	18.5	30	7.78	6.8	18.0	30
6.25	1	8.02	7.4	20.4	29	7.78	4.6	19.2	30	7.62	5.2	19.2	30	7.72	6.2	18.6	30	7.72	6.6	18.1	31
	2					7.70	4.5	19.2	30	7.57	5.2	19.1	30	7.68	6.0	18.5	30	7.69	6.4	18.0	31
	3					7.72	4.5	19.2	30	7.58	5.2	19.2	30	7.69	6.0	18.6	30	7.70	6.4	18.1	31
12.5	1	8.03	7.5	20.4	29	7.71	4.5	19.2	30	7.50	4.4	19.2	30	7.66	5.8	18.6	30	7.67	6.4	18.0	31
	2					7.72	4.5	19.2	30	7.41	4.0	19.2	30	7.59	5.8	18.6	30	7.60	6.0	18.0	31
	3					7.77	4.4	19.3	30	7.38	3.9	19.2	30	7.56	5.6	18.6	30	7.58	6.0	18.2	31
25	1	8.03	7.5	20.3	29	7.80	4.6	19.3	30	7.37	2.7	19.2	30	7.47	4.5	18.7	30	7.51	5.6	18.2	30
	2					7.70	4.4	19.2	30	7.31	2.0	19.2	30	7.47	4.6	18.6	30	7.57	5.7	18.0	31
	3					7.74	4.4	19.3	30	7.32	2.2	19.3	30	7.43	4.4	18.7	30	7.50	5.6	18.3	31
50	1	8.04	7.6	20.4	29	7.75	4.5	19.3	30	7.28	2.0	19.3	30	7.25	2.2	18.9	30	7.31	3.8	18.4	31
	2					7.77	4.8	19.2	30	7.26	2.0	19.2	30	7.21	1.6	18.9	30	7.18	2.2	18.3	31
	3					7.80	4.8	19.4	30	7.27	2.0	19.4	30	7.22	1.8	18.9	30	7.18	2.4	18.5	31
100	1	8.04	7.6	20.4	29	7.87	5.1	19.4	30	—	—	—	—	—	—	—	—	—	—	—	—
	2					7.79	4.4	19.3	30	—	—	—	—	—	—	—	—	—	—	—	—
	3					7.82	4.7	19.4	30	7.17	0.4	19.4	30	—	—	—	—	—	—	—	—
Min		8.00	7.4	20.3	29	7.70	4.4	19.1	30	7.17	0.4	19.1	30	7.21	1.6	18.5	30	7.18	2.2	18.0	30
Max		8.04	7.6	20.4	29	7.91	6.0	19.4	30	7.80	6.2	19.4	30	7.81	6.3	18.9	30	7.78	6.8	18.5	31

Note: — = All animals dead.

Advanced Biological Testing Inc.

TABLE 6

Penaeus vannamei

SURVIVAL DATA FOR REFERENCE TOXICANT (S.D.S.) TEST

Concentration (mg/L)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
6.25	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
12.5	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
25	1	10	10	9	10	10	100	100.0
	2	10	10	8	10	10	100	
	3	10	10	10	10	10	100	
50	1	10	9	9	3	3	30	30.0
	2	10	10	10	8	6	60	
	3	10	10	10	3	0	0	
100	1	10	0	—	—	—	0	0.0
	2	10	0	—	—	—	0	
	3	10	1	0	—	—	0	

Note: — = All animals dead.

LC50 = 42.9 mg/L.

Laboratory mean = 26.39 mg/L.

REFERENCES

U.S. EPA. 1991. Methods for measuring acute toxicity of effluents to freshwater and marine organisms, 4th ed. EPA 600/4-90/027, September, 1991.

TECHNICAL MEMORANDUM**CH2M HILL**

PREPARED FOR: StarKist Samoa, Inc.
VCS Samoa Packing Company, Inc.

PREPARED BY: Steve Costa/CH2M HILL/SFO
Karen A. Glatzel/Glatzel & Associates

DATE: 9 August 1996

SUBJECT: Bioassay Testing of Effluent
February 1996 (Delayed Fall Sept/Oct 1995) Sampling

PROJECT: 107091.EL.96

Purpose

This memorandum presents the results of the effluent bioassay testing of the Joint Cannery Outfall effluent sample that was collected in February 1996. This is the sixth of the required semi-annual tests. Separate technical memoranda describe the results of concurrent effluent chemistry testing. The February 1996 bioassay test is considered to replace the delayed Fall (Sept/Oct) 1995 test.

The test initially planned for October 1995 was delayed as a result of problems encountered in shipping laboratory sample containers for the required concurrent effluent chemistry tests. International Air Transportation Association (IATA) regulations for the shipment of dangerous goods were revised in the Fall of 1995. The nitric acid preservative used in the metals chemistry bottles falls under these regulations, but an exception based on volume should have applied. The new regulations were confusing for all air cargo shippers using commercial carriers and shipments were refused by the commercial air carrier from Honolulu to American Samoa. Multiple shipments of sample bottles were attempted using FEDEX, DHL, and AIRBORNE EXPRESS.

Alternative shipping through a private air cargo and container ship was delayed because of schedule delays between December 1995 and late January 1996 of these transporters. Sample containers were shipped via Triple B Packers at the end of January. The canneries collected the samples within one week of the arrival of the sample containers. Attempts to avoid future delays will be made by early shipping and stockpiling sample collection kits in Samoa in advance of the tests. Although the canneries have little storage space available, an attempt will be made to secure an area for sample containers in the future.

Study Objectives

Section D.1 of the StarKist Samoa and VCS Samoa Packing NPDES permits requires that semi-annual definitive acute bioassays (96-hour static bioassays) be conducted on the

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cannery effluent. The purpose of these bioassays is to determine whether, and at what effluent concentration, acute toxicity may be detected for the effluent.

U.S. EPA has conducted a number of reviews of the effluent sampling, analysis, and bioassay tests. All comments from U.S. EPA have been incorporated into either the Standard Operating Procedures or have been incorporated into the procedures used by the laboratory doing the test, Advanced Biological Testing, Inc., as documented in the March 1995 Technical Memorandum.

The bioassays were originally specified to be conducted using the white shrimp, *Penaeus vannamei* (postlarvae). In the event *Penaeus vannamei* are not available at the time of the tests, a substitute species, *Mysidopsis bahia*, has been approved by U.S. EPA. Prior to the March 1995 test there was evidence that *Penaeus vannamei* would not be available. However, a source of this organism was found. Since the mysids had already been ordered, bioassays were conducted with both *Penaeus vannamei* and *Mysidopsis bahia*. The initial lack of availability of *Penaeus vannamei* in the previous test had resulted in the same situation. Both tests have provided an opportunity to have a side-by-side test using the two organisms and provide information for the evaluation of the overall bioassay testing study since previous tests have been run with each species and substitution may occur with future tests.

The acute bioassay effluent sampling must be concurrent with effluent sampling for priority pollutant chemical analysis. Effluent samples are to be collected as 24-hour composite samples. The effluent acute bioassay was conducted using a combined composite effluent sample made up from the composite effluent samples from the StarKist Samoa and VCS Samoa Packing facilities, as approved by EPA. This combined effluent bioassay is representative of the wastewater discharged from the joint cannery outfall to Pago Pago Harbor.

Effluent Sampling Methods

Between 1200 on February 01 and 0900 on February 02, 1996, 24-hour, flow-weighted, composite samples of final effluent were collected from both the StarKist Samoa and VCS Samoa Packing treatment plant discharges. Samples were collected from the established effluent sampling sites following the routine composite sample collection schedule for the plants. Detailed sampling procedures were provided in the March 1995 technical memorandum as Attachment II.

A total of eight grab samples were collected into pre-cleaned 1-gallon plastic cubitainers at each plant. Samples were collected at approximately three-hour intervals over a 24 hour

period. The samples were stored on ice until the completion of the 24-hour sampling period. After all samples were collected a flow-proportioned composite sample was prepared. The grab sample collection times and the relative effluent volumes calculated from plant flow records are summarized in Table 1. The relative effluent volumes were used to prepare the final composite sample, which was used to fill the sample container shipped to the laboratory for testing.

A 5-gallon cubitainer containing the composite sample was packed on ice in an ice chest for shipment to the laboratory. Sample chain of custody forms were completed and then sealed into zip-lock bags and taped inside the lid of the ice chest. Samples were shipped via DHL on flights from Pago Pago to Honolulu and then to San Francisco. Samples were delivered to the testing laboratory on 5 February 1996.

Bioassay Testing Procedures

The bioassay tests were conducted by Advanced Biological Testing Inc., Tiburon, California. The testing procedures and results of the bioassay tests are provided in "*Results of a Bioassay Conducted on an Effluent Sample from the Joint Cannery Outfall in American Samoa using Penaeus vannamei and Mysidopsis bahia*" dated 1 April 1996 and included as Attachment I. This report summarizes the 96-hour acute bioassay test conducted with reference to U.S. EPA (1991) document *EPA/600/4-90/027* as the source of methods for conducting the test.

The bioassay tests were conducted considering and including U.S. EPA's comments on previous bioassay tests. A brine control was run and a comparison was made with the dilution water "laboratory control". The test organisms were required to be 1 to 5 days old, with a 24-hour range in age and that test temperature be 20 ± 1 °C or 25 ± 1 °C. The mysids were 3-day old larvae tested at 25 ± 2 °C and the penaeids were postlarvae (8 to 10 mm) tested at 20 ± 2 °C.

Because of the demonstrated potential for a lethal immediate dissolved oxygen demand (IDOD), discussed and documented in previous technical memoranda describing the first two bioassay tests, each bioassay test chamber was continuously aerated during the bioassay tests to maintain adequate levels of dissolved oxygen (DO). Bioassay tests were carried out for effluent concentrations of 50, 25, 12.5, 6.25, and 3.1% for both species as vol:vol dilutions in seawater. Water quality was monitored daily with parameters measured including DO, pH, salinity, temperature, and ammonia. A reference toxicant of sodium dodecyl sulfonate (SDS) was made up of a 2-gram per liter stock solution in distilled water and run at concentrations of 100, 50, 25, 12.5, and 6.25 mg/L in 31 ppt seawater for a 96-hour test.

Results

The results of the bioassay tests are summarized as follows:

***Penaeus vannamei* Effluent Bioassay.** All results from the bioassay tests are included in Attachment I. The results of the penaeid 96-hour bioassay indicate the LC_{50} for the effluent tested was >50 percent. The No Observable Effects Concentration (NOEC) for the 96-hour bioassay was >50 percent and the Least Observable Effects Concentration (LOEC) was >50 percent. The calculated value of toxicity unit (TU) was <2.

***Penaeus vannamei* Reference Toxicant Bioassay.** The reference toxicant had a LC_{50} of 24 mg/l. The laboratory mean was 21.59 mg/l and the data was slightly over one standard deviation from the laboratory mean, indicating normal sensitivity.

***Mysidopsis bahia* Effluent Bioassay.** All results from the bioassay are included in Attachment I. The results of the mysid 96-hour bioassay tests indicate the LC_{50} for the effluent tested was 28.36 percent (95 percent confidence limits = 21.4 percent to 35.2 percent). The NOEC for the 96-hour bioassay was 12.5 percent and the LOEC was 25 percent. The calculated value of TU was 8.

***Mysidopsis bahia* Reference Toxicant Bioassay.** The reference toxicant had a LC_{50} of 18.3 mg/l. The laboratory mean was 14.29 mg/l, with a standard deviation of 4.11 mg/l. The data was within one standard deviation of the laboratory mean, indicating normal sensitivity.

Discussion

Table 2 summarizes the results of the effluent bioassay tests for the samples collected in the February 1996 sampling compared to the previous bioassay tests. The NOEC and LC_{50} are higher than those obtained for the October 1993, February 1994, and March 1995 penaeid tests. The higher LC_{50} and NOEC for mysids in the test is intermediate between the results for the previous March 95 and October 94 tests.

Conclusions

The results of the bioassay tests for the Joint Cannery Outfall effluent for February 1996 are not considered to be of concern. As discussed in the reports for the previous tests on this effluent, the time scale of the mixing of the effluent with the receiving water is on the order

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The results of the bioassay tests for the Joint Cannery Outfall effluent for February 1996 are not considered to be of concern. As discussed in the reports for the previous tests on this effluent, the time scale of the mixing of the effluent with the receiving water is on the order of minutes to seconds to achieve dilutions that will eliminate possible toxic effects as reflected by the bioassay results. For example an NOEC of 12.5% corresponds to a dilution of 8:1, and a NOEC of >50% corresponds to a dilution of <2:1, which is achieved in a few seconds. The discharge is located in about 180 feet of water and the effluent toxicity tests indicate that the discharge is diluted to non-toxic levels immediately after discharge and the dilution needed to achieve this is well within the initial dilution plume of the discharge.

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Table 1 StarKist Samoa and VCS Samoa Packing 24-hour Composite Effluent Sample for Bioassay Testing 01-02 February 1996						
Grab Sample Number	VCS Samoa Packing		StarKist Samoa		VCS Sa- moa Packing Percent of Total Flow	StarKist Samoa Percent of Total Flow
	Sampling Date and Time	Effluent Flow Rate (mgd)	Sampling Date and Time ¹	Effluent Flow Rate (mgd)		
1	2/1/96 1200	0.54	2/1/96 1200	1.19	3.7	8.1
2	1500	0.52	1500	1.33	3.6	9.0
3	1800	0.48	1800	1.75	3.3	12.0
4	2100	0.46	2100	0.84	3.1	5.7
5	2400	0.58	2400	1.05	4.0	7.2
6	2/2/96 0300	0.58	2/2/96 0300	1.30	4.0	8.9
7	0600	0.52	0600	1.68	3.6	11.5
8	0900	0.54	0900	1.26	3.7	8.6
Total		4.22		10.4	29.0	71.0
Mean		0.53		1.30		
¹ Approximate sampling times as exact times not recorded						

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Table 2 StarKist Samoa and VCS Samoa Packing Combined Effluent Bioassay Results				
Date	Species	Parameters		
		LC 50	NOEC	LOEC
2/93	<i>Penaeus vannami</i>	4.8% ¹	3.1 %	6.25 %
10/93	<i>Penaeus vannami</i>	15.67 %	3.1 %	6.25 %
2/94	<i>Penaeus vannami</i>	15.76 %	< 1.6 %	1.6 %
10/94	<i>Mysidopsis bahia</i> ²	31.2 %	25 %	50 %
3/95	<i>Penaeus vannami</i>	14.8 %	6.25 %	12.5 %
3/95	<i>Mysidopsis bahia</i> ³	10.8 %	6.25 %	12.5 %
2/96	<i>Penaeus vannami</i>	> 50 %	> 50 %	> 50 %
2/96	<i>Mysidopsis bahia</i> ³	28.36 %	12.5 %	25 %
¹ The February 1993 samples were not aerated until after the first day of the test. For subsequent tests the samples were aerated for the entire duration of the tests. ² <i>Mysidopsis bahia</i> substitutes as <i>Penaeus vannami</i> not available, as directed by U. S. EPA. ³ <i>Mysidopsis bahia</i> used in addition to <i>Penaeus vannami</i> as described in text. Only one species is required by the permit conditions.				

ATTACHMENT I

LABORATORY REPORT
Advanced Biological Testing
96-hour Acute Bioassay

JOINT CANNERY OUTFALL EFFLUENT SAMPLE
February 1-2, 1996

**RESULTS OF BIOASSAYS CONDUCTED ON
AN EFFLUENT SAMPLE
FROM THE JOINT CANNERY OUTFALL
IN AMERICAN SAMOA**
Using *Penaeus vannamei* and *Mysidopsis bahia*

Prepared for:

CH2M Hill California, Inc.
1111 Broadway
Oakland, CA 94607
Project # PDX 30702

Prepared by:

Advanced Biological Testing Inc.
98 Main St., # 419
Tiburon, Ca. 94920

April 1, 1996

Ref: 9605-1

INTRODUCTION

At the request of CH2M Hill (Project # PDX 30702), Advanced Biological Testing conducted a four day effluent bioassay test on *Mysidopsis bahia* and *Penaeus vannamei* using effluents collected from the joint cannery outfall at the Starkist and Van Camp tuna canneries in American Samoa. The studies were run using methods generally specified in EPA 1991. *Penaeus* is the preferred species according to the NPDES permit, however in previous studies when *Penaeus* was unavailable, *Mysidopsis* was substituted. Since both species have been tested previously separately, it was decided to continue with both species in this test.

The study was conducted at the Advanced Biological Testing Laboratory in Tiburon, California, and was managed by Mr. Mark Fisler.

2.1 EFFLUENT SAMPLING

The effluents were sampled on February 2, 1996 by cannery personnel under the supervision of CH2M Hill. The sample was received by the laboratory on February 5, 1996. One five gallon carboy was provided and maintained in an ice-filled cooler from the date of sampling until laboratory receipt. The sample was at 5°C upon receipt.

2.2 SAMPLE PREPARATION

The salinity of the effluent sample was 14 ppt and required salinity adjustment to 30 ppt. The effluent salinity was increased to 30 ppt with 100 ppt natural seawater brine. The brine was made from frozen Bodega Bay seawater. Due to the dilution of the effluent with the brine solution, the initial maximum concentration of effluent was 80%. The highest initial test concentration was made by diluting the 80% effluent with Bodega Bay seawater to an actual effluent concentration of 50%. The initial total ammonia was approximately 6 ppm (2.98 ppm in the 50% test sample).

The effluents were tested at an actual effluent concentration series of 50%, 25%, 12.5%, 6.25%, and 3.1% for both species as vol:vol dilutions in seawater. A brine control was run with both test sets to assess the potential toxicity from the added brine. The diluent and the control water was filtered seawater from Bodega Bay. The dilutions were brought to the test temperatures (20 and $25 \pm 2^{\circ}\text{C}$) and aerated continuously. Based upon previous testing, these effluents have an increasing biological oxygen demand, with a significant peak at 10-14 hours after test initiation. Previous testing of this effluent conducted without aeration demonstrated significant toxicity at 24 hours (or before); therefore aeration was carried out from the beginning of the test. According to EPA methods the test chambers were renewed with retained effluents held under refrigeration from test initiation on Day 2.

A reference toxicant was run using concentrations initially provided by the EPA. The toxicant was sodium dodecyl sulfonate (SDS) made up as a 2 grams per liter stock solution in distilled water. The tested concentrations were set at 100, 50, 25, 12.5 and 6.25 mg/L in 31 ppt seawater.

2.3 TESTING PROCEDURES

The bioassays were carried out on three day old larvae of *Mysidopsis bahia* supplied by Aquatox in Arkansas and post-larval *Penaeus* provided by Brezina and Associates. The mysids were received on February 7, 1996 and the penaeids on February 6, 1996. Five replicates of each concentration were tested with ten animals per replicate. Water quality was monitored daily as initial quality on Day 0 and final water quality on Days 1-4. Parameters measured included dissolved oxygen, pH, salinity, total ammonia, and temperature.

2.4 STATISTICAL ANALYSIS

At the conclusion of the test, the survival data were evaluated statistically using ToxCalc™ to determine ECp, NOEC, and LOEC values where appropriate. ToxCalc™ is a comprehensive statistical application that follows standard guidelines for acute toxicity data analysis. Statistical effects can be measured by the ECp, the estimated concentration that causes any effect, either lethal (LC) or sublethal (IC), on p% of the test population. The LCp is the point estimate of the concentration at which a lethal effect is observed in p% of the test organisms. ECp values include 95% confidence limits if calculable. The Toxicity units (TU) are defined as 100/NOEC.

3.1 INTRODUCTION

Tables 1 through 6 present the data for the *Penaeus* testing and Tables 7 through 12 present the results of the *Mysidopsis* testing.

3.2 *Penaeus vannamei*

The test conditions are summarized in Table 1. Water quality measurements were within the acceptable limits provided in EPA 1991. Temperature was maintained at $20 \pm 2^{\circ}\text{C}$; pH remained relatively stable, and the salinity increased slightly as would be expected in a static test (Tables 2 and 3). Aeration was maintained in all chambers for the duration of the test. The test solutions were renewed with reserved effluent at 48 hrs.

Initial ammonia was 2.98 ppm in the 50% effluent and was proportionally diluted at lower percentage concentrations. At the end of the test the 50% concentration had risen slightly to 3.54 ppm and the lower concentrations were again proportional.

There was little toxicity observed in the penaeid test, with 86% survival in the 50% concentration (Table 4). The LC50 for the effluent was >50%. The NOEC was >50%. The TU was <2.

The reference toxicant test had an LC50 of 24 mg/L (Tables 5 and 6). The laboratory mean was 21.59 mg/L and the data was slightly over one standard deviation from the laboratory mean, indicating normal sensitivity.

3.2 *Mysidopsis bahia*

The test conditions are summarized in Table 7. Water quality measurements were within the acceptable limits provided in EPA 1991. Temperature was maintained at $25 \pm 2^{\circ}\text{C}$; and the pH remained relatively stable, and the salinity increased very slightly as would be expected in a static test (Tables 8 and 9). Aeration was maintained in all chambers for the duration of the test. The test solutions were renewed with reserved effluent at 48 hrs.

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Initial ammonia was 2.98 ppm in the 50% effluent and was proportionally diluted at lower percentage concentrations. At the end of the test the 25% concentration had risen slightly from 1.34 ppm on Day 0 to 1.81 ppm. The lower concentrations were proportional.

The LC50 for the effluent was 28.36% (95% confidence limits = 21.4% to 35.2%). There was significant mortality at the 25% and 50% concentrations compared to the control (Table 10). The NOEC was 12.5%, and the LOEC was 25%. The TU was 8.

The reference toxicant test had an LC50 of 18.3 mg/L (Tables 11 and 12). The laboratory mean for *Mysidopsis bahia* was 14.29 mg/L (SD = 4.11 mg/L). The data is within one standard deviation of the laboratory mean, indicating normal sensitivity.

TABLE 1

Bioassay Procedure And Organism Data
For the Survival Bioassay
Using *Penaeus vannamei* U.S. EPA 1991)

Parameter	Data
<u>Sample Identification</u>	
Sample ID(s)	960205-1
Date Sampled	2/2/96
Date Received at ABT	2/5/96
Volume Received	Five gallons
Sample Storage Conditions	4°C in the dark
<u>Test Species</u>	
Supplier	J. Brezina and Associates
Collection location	Hawaii
Date Acquired	2/6/96
Acclimation Time	24 hours
Acclimation Water	Shipping water
Acclimation Temperature	20±2°C
Age group	Post larvae (approximately 8-10 mm)
<u>Test Procedures</u>	
Type; Duration	Acute, static/renewal at 48 hours
Test Dates	2/7-11/96
Control Water	Bodega Bay seawater
Test Temperature	20 ± 2°C
Test Photoperiod	16L : 8 D
Salinity	30± 2 ppt
Test Chamber	1000 mL jars
Animals/Replicate	10
Exposure Volume	500 mL
Replicates/Treatment	5
Feeding	Brine shrimp (24 hr old nauplii)
Deviations from procedures	Aerated continuously

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TABLE 2

Penaeus vannamei
INITIAL WATER QUALITY MEASUREMENTS
FOR EFFLUENT TEST
Initial Readings

Concentration (%)	Day 0					Day 2				
	pH	DO	NH 3	°C	Sal	pH	DO	NH 3	°C	Sal
Control	8.00	8.0	0.01	18.0	30	8.08	7.6	0.01	19.0	30
Brine	8.10	7.9	0.01	18.0	30	7.99	8.4	0.01	20.1	30
3.1	7.98	7.9	0.17	18.0	30	7.71	4.8	0.17	19.5	30
6.25	7.98	7.8	0.35	18.0	30	7.77	6.6	0.34	19.4	30
12.5	7.84	7.6	0.70	18.0	30	7.65	5.2	0.63	19.2	30
25	7.84	7.6	1.34	18.0	30	7.56	4.8	1.21	19.4	30
50	7.80	7.2	2.98	18.0	29	—	—	—	—	—
Min	7.80	7.2	0.01	18.0	29	7.56	4.8	0.01	19.0	30
Max	8.10	8.0	2.98	18.0	30	8.08	8.4	1.21	20.1	30

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TABLE 3

Penaeus vannamei

FINAL WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST

Concentration (%) Rep		Day 1				Day 2				Day 3				Day 4				
		pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	NH 3	°C	Sal
Control	1	8.06	7.6	19.2	30	8.08	7.4	19.6	30	8.21	6.3	19.0	30	8.16	7.1	0.09	19.6	30
	2	8.12	7.6	19.1	30	8.14	7.4	19.5	30	8.26	6.1	19.2	30	8.23	7.2		19.8	30
	3	8.09	7.6	19.1	30	8.07	7.4	19.5	30	8.16	6.0	19.1	30	8.10	7.3		19.7	30
	4	8.11	7.6	19.1	30	8.12	7.4	19.4	30	8.24	6.1	19.1	30	8.20	7.1		19.6	30
	5	8.12	7.6	19.1	30	8.13	7.4	19.4	30	8.25	6.0	19.1	30	8.20	7.2		19.6	30
Brine Control	1	8.23	7.6	19.2	30	8.24	7.5	19.6	30	8.27	6.3	19.2	30	8.37	7.3	0.07	19.9	31
	2	8.23	7.6	19.1	30	8.23	7.4	19.4	30	8.37	6.3	19.2	30	8.34	7.1		19.7	30
	3	8.25	7.6	19.1	30	8.25	7.4	19.4	30	8.38	6.2	19.2	30	8.35	7.4		19.6	30
	4	8.25	7.6	19.0	30	8.26	7.4	19.3	30	8.38	6.2	19.1	30	8.36	7.2		19.6	31
	5	8.26	7.6	19.0	30	8.27	7.4	19.3	30	8.39	6.1	19.1	30	8.36	7.2		19.6	31
3.1	1	8.14	7.6	19.1	30	8.13	7.4	19.5	30	8.25	6.4	19.2	30	8.22	7.2	0.37	19.9	30
	2	8.16	7.6	19.0	30	8.17	7.4	19.3	31	8.30	6.2	19.1	31	8.29	7.2		19.7	31
	3	8.18	7.6	19.0	30	8.18	7.4	19.3	31	8.30	6.4	19.1	31	8.28	7.2		19.6	31
	4	8.12	7.6	19.0	30	8.13	7.4	19.3	30	8.25	6.1	19.1	30	8.21	7.0		19.6	31
	5	8.16	7.6	19.0	30	8.17	7.4	19.3	31	8.24	6.3	19.1	31	8.27	7.0		19.6	31
6.25	1	8.15	7.6	19.1	30	8.14	7.4	19.5	30	8.26	6.1	19.2	30	8.26	7.2	0.57	19.9	30
	2	8.12	7.6	19.1	30	8.11	7.4	19.4	30	8.23	6.0	19.2	30	8.22	7.0		19.7	31
	3	8.15	7.6	19.0	30	8.17	7.4	19.3	30	8.28	6.1	19.1	31	8.28	7.2		19.6	31
	4	8.14	7.6	19.1	30	8.16	7.4	19.3	30	8.27	6.1	19.1	30	8.29	7.2		19.6	31
	5	8.15	7.6	19.1	30	8.15	7.4	19.3	30	8.25	6.0	19.1	30	8.12	7.1		19.6	31
12.5	1	8.07	7.6	19.2	30	8.04	7.2	19.5	30	8.18	6.0	19.2	30	8.22	7.0	1.00	19.9	30
	2	8.16	7.6	19.1	30	8.15	7.2	19.4	30	8.30	6.0	19.1	31	8.24	7.1		19.7	30
	3	8.13	7.6	19.1	30	8.14	7.2	19.4	30	8.29	5.9	19.1	30	8.19	7.2		19.8	30
	4	8.10	7.6	19.1	30	8.08	7.0	19.4	30	8.27	5.9	19.1	30	8.27	7.2		19.8	30
	5	8.10	7.6	19.1	30	8.10	7.0	19.4	30	8.23	6.0	19.2	30	8.26	7.0		19.6	30
25	1	8.11	7.6	19.1	30	8.10	7.2	19.5	30	8.28	6.0	19.1	30	8.33	7.0	1.81	19.8	31
	2	8.18	7.4	19.0	30	8.17	7.2	19.3	30	8.33	6.1	19.1	31	8.36	7.0		19.7	32
	3	8.09	7.4	19.1	30	8.09	7.2	19.4	30	8.22	6.0	19.1	30	8.31	7.1		19.7	30
	4	8.05	7.4	19.1	30	8.06	7.2	19.4	30	8.01	6.0	19.2	30	8.29	6.8		19.7	30
	5	8.06	7.4	19.1	30	8.06	7.2	19.5	30	8.30	6.1	19.2	30	8.31	6.8		19.7	30
50	1	8.13	7.2	19.3	30	8.19	7.2	19.6	30	8.36	6.1	19.2	31	8.43	6.4	3.54	19.9	31
	2	8.13	7.0	19.2	30	8.21	7.2	19.5	30	8.30	6.0	19.2	30	8.45	6.8		19.8	31
	3	8.19	7.2	19.2	30	8.25	7.2	19.5	30	8.41	5.9	19.2	31	8.45	6.8		19.9	31
	4	8.15	7.2	19.4	30	8.17	7.0	19.8	30	8.37	6.0	19.4	30	8.33	6.7		19.9	31
	5	8.17	7.1	19.5	30	8.20	7.0	19.9	30	8.42	6.0	19.4	31	8.44	6.7		19.7	31
Min		8.05	7.0	19.0	30	8.04	7.0	19.3	30	8.01	5.9	19.0	30	8.10	6.4	0.07	19.6	30
Max		8.26	7.6	19.5	30	8.27	7.5	19.9	31	8.42	6.4	19.4	31	8.45	7.4	3.54	19.9	32

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TABLE 4

Penaeus vannamei
SURVIVAL DATA FOR EFFLUENT TEST

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
Brine Control	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
3.1	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
6.25	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
12.5	1	10	10	10	10	10	100	98.0
	2	10	10	9	9	9	90	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
25	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
50	1	10	10	10	10	9	90	86.0
	2	10	10	10	10	9	90	
	3	10	10	10	10	9	90	
	4	10	9	9	9	6	60	
	5	10	10	10	10	10	100	

TABLE 5

Penaeus vannamei
WATER QUALITY MEASUREMENTS
FOR REFERENCE TOXICANT (S.D.S) TEST

Concentration (mg/L)	Rep	Day 0				Day 1				Day 2				Day 3				Day 4			
		pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal
Control	1	8.01	8.0	19.4	30	7.91	6.8	19.4	30	7.93	6.4	19.7	30	7.91	4.5	19.4	30	7.94	5.0	20.1	31
	2					7.93	6.8	19.3	30	7.95	6.4	19.6	30	7.99	4.6	19.3	31	7.93	5.0	19.9	31
	3					7.93	6.8	19.4	30	7.94	6.4	19.7	30	7.98	4.4	19.4	31	7.91	5.0	19.9	31
6.25	1	8.01	8.0	19.1	30	7.76	4.8	19.4	30	7.86	6.0	19.7	30	7.95	4.4	19.5	31	7.88	4.5	20.1	31
	2					7.71	4.8	19.3	30	7.82	6.0	19.6	30	7.97	4.5	19.4	31	7.95	4.8	19.9	31
	3					7.72	4.6	19.4	30	7.83	5.8	19.7	30	7.95	4.1	19.4	31	7.85	4.2	20.0	31
12.5	1	8.02	8.0	19.1	30	7.57	4.2	19.4	30	7.72	5.2	19.7	30	7.80	3.2	19.5	31	7.79	4.0	20.1	31
	2					7.54	4.2	19.4	30	7.69	5.2	19.6	30	7.78	3.2	19.4	30	7.77	4.0	20.1	31
	3					7.54	4.2	19.4	30	7.69	5.1	19.7	30	7.78	3.4	19.4	31	7.80	4.0	20.1	31
25	1	8.02	8.0	19.1	30	7.43	3.9	19.5	30	7.47	4.8	19.9	30	7.61	2.4	19.5	31	7.70	3.5	20.3	31
	2					7.43	3.9	19.4	30	7.46	4.7	19.7	30	7.62	2.5	19.4	31	7.73	3.7	20.2	31
	3					7.44	3.7	19.5	30	7.47	4.7	19.8	30	7.64	2.5	19.4	31	7.73	3.5	20.2	31
50	1	8.02	8.0	19.2	30	7.46	4.0	19.6	30	7.39	4.2	19.9	30	7.40	0.1	19.5	31	7.50	1.4	20.1	31
	2					7.50	4.0	19.5	30	7.36	4.4	19.8	30	7.36	0.1	19.5	31	7.46	0.8	20.3	31
	3					7.48	4.0	19.6	30	7.37	4.3	19.9	30	7.38	0.1	19.5	31	7.47	0.5	20.3	31
100	1	8.02	8.0	19.7	30	7.45	3.8	19.6	30	—	—	—	—	—	—	—	—	—	—	—	—
	2					7.41	3.7	19.6	30	—	—	—	—	—	—	—	—	—	—	—	—
	3					7.43	3.5	19.7	30	—	—	—	—	—	—	—	—	—	—	—	—
Min		8.01	8.0	19.1	30	7.41	3.5	19.3	30	7.36	4.2	19.6	30	7.36	0.1	19.3	30	7.46	0.5	19.9	31
Max		8.02	8.0	19.7	30	7.93	6.8	19.7	30	7.95	6.4	19.9	30	7.99	4.6	19.5	31	7.95	5.0	20.3	31

Note: — = All animals dead.

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TABLE 6

Penaeus vannamei
SURVIVAL DATA FOR REFERENCE TOXICANT (S.D.S.) TEST

Concentration (mg/L)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
6.25	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
12.5	1	10	10	10	10	10	100	96.7
	2	10	10	9	9	9	90	
	3	10	10	10	10	10	100	
25	1	10	10	7	7	5	50	30.0
	2	10	7	3	3	1	10	
	3	10	7	3	3	3	30	
50	1	10	6	2	2	2	20	26.7
	2	10	6	4	4	3	30	
	3	10	6	3	3	3	30	
100	1	10	0	—	—	—	0	0.0
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	

Note: — = All animals dead.

LC50 = 24 mg/L.

Laboratory mean = 21.59 mg/L.

TABLE 7

**Bioassay Procedure And Organism Data
For the Survival Bioassay
Using *Mysidopsis bahia* (U.S. EPA 1991)**

<u>Parameter</u>	<u>Data</u>
<u>Sample Identification</u>	
Sample ID(s)	960205-1
Date Sampled	2/2/96
Date Received at ABT	2/5/96
Volume Received	Five gallons
Sample Storage Conditions	4°C in the dark
<u>Test Species</u>	
Supplier	Aquatox, Hot Springs, Arkansas
Collection location	In house colony
Date Acquired	2/7/96
Acclimation Time	Used immediately
Acclimation Water	Shipping water
Acclimation Temperature	25±2°C
Age group	Three day old larvae
<u>Test Procedures</u>	
Type; Duration	Acute, static/renewal at 48 hours
Test Dates	2/7-11/96
Control Water	Bodega Bay seawater
Test Temperature	25± 2°C
Test Photoperiod	14 L : 10 D
Salinity	30± 2 ppt
Test Chamber	1000 mL jars
Animals/Replicate	10
Exposure Volume	500 mL
Replicates/Treatment	5
Feeding	Brine shrimp (<24 hr old nauplii)
Deviations from procedures	None

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TABLE 8

Mysidopsis bahia
INITIAL WATER QUALITY MEASUREMENTS
FOR EFFLUENT TEST
Initial Readings

Concentration (%)	Day 0					Day 2			
	pH	DO	NH 3	°C	Sal	pH	DO	°C	Sal
Control	8.00	8.0	0.01	24.2	30	8.09	7.6	24.6	30
Brine	8.10	7.9	0.01	24.2	30	7.98	7.6	24.4	30
3.1	7.98	7.9	0.17	24.3	30	7.67	5.0	24.6	30
6.25	7.98	7.8	0.35	24.2	30	7.69	6.1	24.3	30
12.5	7.84	7.6	0.70	24.3	30	7.64	4.6	24.9	30
25	7.84	7.6	1.34	24.2	30	7.57	4.2	24.9	30
50	7.80	7.2	2.98	24.2	29	—	—	—	—
Min	7.80	7.2	0.01	24.2	29	7.57	4.2	24.3	30
Max	8.10	8.0	2.98	24.3	30	8.09	7.6	24.9	30

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TABLE 9

Mysidopsis bahia FINAL WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST

Concentration (%)	Rep	Day 1				Day 2				Day 3				Day 4				
		pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	NH 3	°C	Sal
Control	1	8.20	7.0	25.3	31	8.20	6.4	25.3	32	8.26	5.8	25.4	31	8.28	6.8	0.09	26.0	32
	2	8.20	6.8	25.3	31	8.21	6.5	25.3	32	8.29	6.0	25.5	31	8.28	6.7		26.1	32
	3	8.19	6.8	25.3	31	8.16	6.5	25.4	32	8.23	5.8	25.6	31	8.22	6.6		26.0	32
	4	8.14	6.8	25.4	31	8.14	6.4	25.4	32	8.17	5.9	25.6	31	8.19	6.5		26.0	32
	5	8.19	6.8	25.6	31	8.21	6.4	25.5	32	8.27	6.0	25.6	31	8.30	6.5		26.0	32
Brine Control	1	8.31	6.8	25.4	31	8.30	6.4	25.4	31	8.39	6.1	25.6	31	8.36	6.5	0.07	26.0	31
	2	8.32	6.8	25.4	31	8.32	6.4	25.4	31	8.37	6.0	25.6	31	8.38	6.4		26.0	31
	3	8.32	6.7	25.4	31	8.32	6.4	25.4	31	8.38	6.0	25.7	31	8.36	6.4		26.1	31
	4	8.30	6.7	25.6	31	8.29	6.4	25.6	31	8.37	6.0	25.7	31	8.38	6.4		26.1	31
	5	8.23	6.6	25.6	31	8.22	6.4	25.6	31	8.25	5.8	25.7	31	8.26	6.4		26.0	31
3.1	1	8.22	6.6	25.5	31	8.21	6.4	25.4	31	8.29	5.9	25.7	31	8.30	6.4	0.32	26.0	31
	2	8.18	6.6	25.6	31	8.18	6.4	25.6	31	8.26	6.0	25.7	31	8.27	6.3		26.1	31
	3	8.22	6.6	25.6	31	8.23	6.4	25.6	31	8.31	6.1	25.8	31	8.30	6.4		26.0	31
	4	8.21	6.6	25.6	31	8.22	6.4	25.6	31	8.28	6.1	25.8	31	8.33	6.4		26.0	31
	5	8.22	6.6	25.7	31	8.23	6.4	25.6	31	8.32	6.0	25.8	31	8.29	6.4		26.0	31
6.25	1	8.23	6.8	25.5	31	8.24	6.4	25.4	31	8.34	6.0	25.7	31	8.35	6.4	0.57	26.1	31
	2	8.20	6.7	25.6	31	8.17	6.4	25.6	31	8.26	6.0	25.9	31	8.31	6.4		26.0	31
	3	8.19	6.6	25.8	31	8.17	6.4	25.6	31	8.25	6.0	25.9	31	8.27	6.4		26.0	31
	4	8.17	6.6	25.8	31	8.16	6.4	25.7	31	8.27	5.8	25.9	31	8.28	6.4		26.0	31
	5	8.22	6.7	25.8	31	8.21	6.4	25.8	31	8.30	6.1	25.9	31	8.32	6.4		26.0	31
12.5	1	8.21	6.6	25.6	31	8.18	6.4	25.6	31	8.28	6.0	25.7	31	8.34	6.4	1.00	26.1	31
	2	8.21	6.6	25.7	31	8.18	6.4	25.6	31	8.31	6.0	25.8	31	8.33	6.4		26.0	31
	3	8.21	6.6	25.8	31	8.22	6.4	25.7	31	8.26	6.0	25.8	31	8.36	6.4		26.0	31
	4	8.19	6.6	25.9	31	8.21	6.4	25.8	31	8.29	5.8	25.9	31	8.35	6.4		26.0	31
	5	8.23	6.6	25.9	31	8.23	6.4	25.8	31	8.27	5.8	25.9	31	8.37	6.4		26.7	31
25	1	8.12	6.4	25.8	31	8.09	6.2	25.7	31	8.16	5.6	25.9	31	8.31	6.2	1.81	26.0	31
	2	8.21	6.4	25.9	31	8.21	6.3	25.8	31	8.29	5.5	25.9	31	8.38	6.2		26.1	31
	3	8.23	6.6	25.8	31	8.26	6.4	25.8	31	8.37	5.5	25.9	31	8.41	6.2		26.1	31
	4	8.24	6.6	25.9	31	8.22	6.4	25.8	31	8.34	5.7	25.9	31	8.40	6.2		26.0	31
	5	8.23	6.5	25.9	31	8.22	6.4	25.9	31	8.32	5.8	25.9	31	8.39	6.2		26.0	31
50	1	8.09	6.2	25.4	31	—	—	—	—	—	—	—	—	—	—	—	—	—
	2	8.07	6.3	25.6	31	—	—	—	—	—	—	—	—	—	—	—	—	—
	3	7.98	6.0	25.5	31	—	—	—	—	—	—	—	—	—	—	—	—	—
	4	7.99	6.0	25.5	31	—	—	—	—	—	—	—	—	—	—	—	—	—
	5	8.27	6.6	25.5	31	8.31	6.4	25.4	32	—	—	—	—	—	—	—	—	—
Min		7.98	6.0	25.3	31	8.09	6.2	25.3	31	8.16	5.5	25.4	31	8.19	6.2	0.07	26.0	31
Max		8.32	7.0	25.9	31	8.32	6.5	25.9	32	8.39	6.1	25.9	31	8.41	6.8	1.81	26.7	32

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TABLE 10

Mysidopsis bahia
SURVIVAL DATA FOR EFFLUENT TEST

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	10	9	90	90.0
	2	10	8	8	8	8	80	
	3	10	10	10	9	9	90	
	4	10	10	10	10	9	90	
	5	10	10	10	10	10	100	
Brine Control	1	10	10	8	8	8	80	92.0
	2	10	10	10	9	9	90	
	3	10	10	10	10	9	90	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
3.1	1	10	10	10	10	10	100	86.0
	2	10	8	7	7	7	70	
	3	10	10	9	8	8	80	
	4	10	10	10	9	8	80	
	5	10	10	10	10	10	100	
6.25	1	10	9	9	9	9	90	92.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	9	90	
	4	10	10	10	10	9	90	
	5	10	10	10	10	9	90	
12.5	1	10	10	7	7	7	70	90.0
	2	10	10	9	9	9	90	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	9	90	
25	1	10	10	10	10	8	80	44.0
	2	10	10	8	8	6	60	
	3	10	10	6	6	4	0	
	4	10	10	6	6	3	30	
	5	10	10	7	7	5	50	
50	1	10	0	—	—	—	0	0.0
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	1	0	—	—	0	

Note: — = All animals dead.

TABLE 11

Mysidopsis bahia
WATER QUALITY MEASUREMENTS
FOR REFERENCE TOXICANT (S.D.S) TEST

Concentration (mg/L)	Rep	Day 0				Day 1				Day 2				Day 3				Day 4			
		pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal
Control	1	8.00	7.6	25.7	30	8.08	6.6	25.4	31	7.94	5.2	25.4	31	7.95	4.2	25.5	31	7.96	5.2	26.2	32
	2					8.08	6.6	25.4	31	7.96	5.4	25.4	31	7.94	4.3	25.6	31	7.95	5.0	26.1	32
	3					8.09	6.6	25.4	31	7.99	5.3	25.4	31	7.95	3.9	25.6	31	7.91	4.6	26.2	32
1.6	1	8.00	7.6	26.0	30	8.06	6.4	25.4	31	7.93	5.0	25.5	31	7.86	3.4	25.6	31	7.91	4.6	26.2	32
	2					8.05	6.4	25.4	31	7.90	4.8	25.5	31	7.94	4.3	25.6	31	7.93	4.8	26.2	32
	3					8.05	6.4	25.4	31	7.92	4.8	25.4	31	7.80	3.0	25.6	31	7.86	4.4	26.1	32
3.1	1	8.00	7.6	26.0	30	8.03	6.2	25.4	31	7.88	4.4	25.5	31	7.86	3.6	25.6	32	7.95	5.0	26.2	32
	2					8.02	6.2	25.4	31	7.93	4.8	25.5	31	7.81	4.0	25.6	31	7.94	5.0	26.3	32
	3					8.02	6.2	25.4	31	7.92	4.6	25.5	31	7.86	3.4	25.6	31	7.89	4.5	26.3	32
6.25	1	8.00	7.6	26.0	30	7.95	5.8	25.6	31	7.86	4.6	25.6	31	7.87	3.8	25.6	32	7.95	4.8	26.2	32
	2					7.94	5.8	25.6	31	7.86	4.6	25.6	31	7.88	3.8	25.6	32	7.95	5.0	26.2	32
	3					7.92	5.5	25.5	31	7.84	4.4	25.4	31	7.81	3.0	25.6	31	7.89	4.6	26.2	32
12.5	1	8.00	7.6	26.0	30	7.77	4.2	25.6	31	7.79	4.6	25.6	31	7.78	3.2	25.6	32	7.84	4.4	26.4	32
	2					7.75	4.2	25.6	31	7.80	4.6	25.6	31	7.78	3.2	25.7	31	7.86	4.4	26.3	32
	3					7.76	4.2	25.6	31	7.85	4.8	25.6	31	7.77	3.0	25.7	31	7.89	4.4	26.3	32
25	1	8.00	7.6	25.9	30	7.62	3.9	25.4	31	7.63	4.2	25.4	31	7.73	3.0	25.4	31	7.78	3.6	26.2	32
	2					7.58	3.9	25.4	31	7.60	4.2	25.4	31	7.75	3.2	25.5	31	7.76	3.6	26.2	32
	3					7.57	3.9	25.4	31	7.55	4.1	25.4	31	7.74	3.4	25.6	31	7.87	5.0	26.2	32
Min		8.00	7.6	25.7	30	7.57	3.9	25.4	31.0	7.55	4.1	25.4	31.0	7.73	3.0	25.4	31.0	7.76	3.6	26.1	32.0
Max		8.00	7.6	26.0	30	8.09	6.6	25.6	31.0	7.99	5.4	25.6	31.0	7.95	4.3	25.7	32.0	7.96	5.2	26.4	32.0

Note: — = All animals dead.

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TABLE 12

Mysidopsis bahia
SURVIVAL DATA FOR REFERENCE TOXICANT (S.D.S.) TEST

Concentration (mg/L)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	10	9	90	90.0
	2	10	10	9	9	9	90	
	3	10	10	9	9	9	90	
1.6	1	10	10	8	8	8	80	90.0
	2	10	10	10	9	9	90	
	3	10	10	10	10	10	100	
3.1	1	10	10	9	8	8	80	86.7
	2	10	10	10	9	9	90	
	3	10	10	10	10	9	90	
6.25	1	10	10	9	9	9	90	90.0
	2	10	10	10	10	10	100	
	3	10	10	8	8	8	80	
12.5	1	10	7	7	7	7	70	66.7
	2	10	7	7	7	7	70	
	3	10	8	6	6	6	60	
25	1	10	2	2	2	2	20	20.0
	2	10	3	3	3	3	30	
	3	10	1	1	1	1	10	

LC50 = 18.3 mg/L.

Laboratory mean = 14.29 mg/L.

REFERENCES

U.S. EPA. 1991. Methods for measuring acute toxicity of effluents to freshwater and marine organisms, 4th ed. EPA 600/4-90/027, September, 1991.